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BEARING ON HEREDITY AND VARIATION

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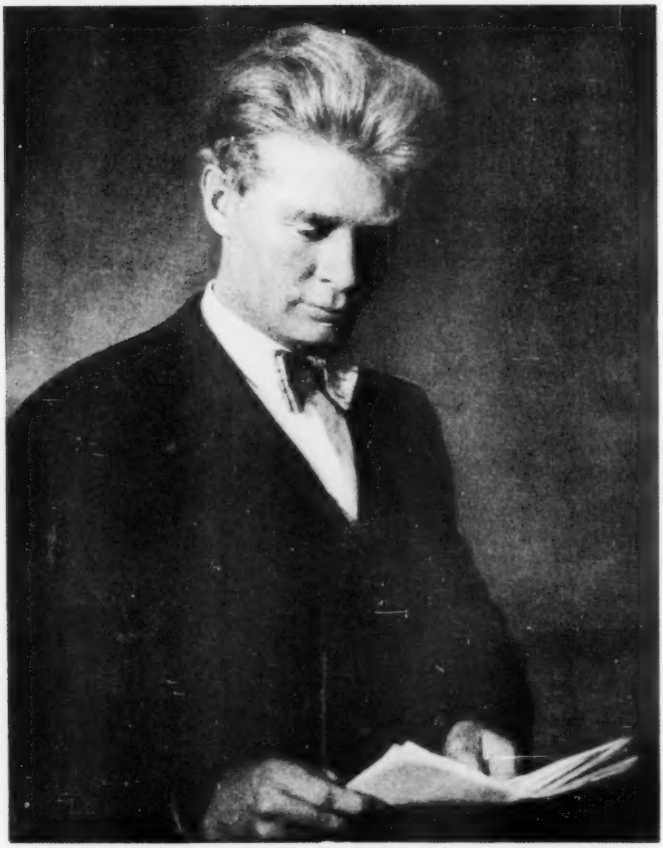

Calvin B. Bridges

CALVIN BLACKMAN BRIDGES

CALVIN BLACKMAN BRIDGES was born in Schuyler's Falls, New York, on January 11, 1889, and died in Los Angeles December 27, 1938. His father, LEONARD VICTOR BRIDGES, who was brought up on a small farm near Plattsburg, married CHARLOTTE AMELIA BLACKMAN in 1887, and Calvin was their only child.

His mother died when Calvin was two years old. His father died a year later. The Grandmother Bridges had already brought up a large family but considered it her duty to take charge of Calvin. He went to a small district school when it was convenient. When he was about 14 years old his grandmother insisted that he should be "educated," and he was allowed to go to Plattsburg. He was not ready for high school and had to spend two preliminary years in grammar school. He graduated from high school in 1909 when he was 20 years old. He had made a fine record. During the years in Plattsburg he drove to school part of the time with neighbors and worked and lived much of the time in town. He worked as "printers devil" on the Plattsburg Press, and it was said "there is a boy who is going to amount to something." When he had spare time he was always at his books, but he was a "regular boy," good-natured and obliging.

One summer during his high school years he worked on a geological survey of Valcour Island with Professor GEORGE H. HUDSON of the Plattsburg Normal School. There is extant a letter by Professor HUDSON written when Calvin applied for a scholarship at Columbia University in which he says: "He is in many respects a very remarkable young man. He has always kept on the honor roll of his school and yet read very extensively outside of the lines of his school work." The list of the books he read, in Calvin's handwriting, exists and it certainly covers a very wide range of subjects. HUDSON also says: "He has the questioning mind, is apt in the forming of hypotheses and quick to see when they are weak. His great desire is to undertake research work."

At the end of his high school work Calvin was urged to try the regional examination for a four year scholarship at Cornell which he passed. He also took an examination for a one year scholarship at Columbia University and passed this also. Wishing to live near his aunt, Mrs. BILLINGS, who lived in New Jersey, he accepted the Columbia scholarship. During his three years at college he received some aid from scholarships, but earned much of his living in other ways. During one of the summers he tutored two young boys and during another he went "on the road" to sell a book entitled "Standard Dictionary of Facts."

My first contact with BRIDGES was in 1909 when he took a course I gave in General Biology and another in Embryology (1910); and later, as a

graduate student, he took my course in Experimental Zoology, largely devoted to genetics, and a course in Experimental Embryology. He attended also Professor E. B. WILSON's course on The Cell.

BRIDGES graduated (B.S.) from Columbia College in 1912. In the same year he married GERTRUDE F. IVES, and is survived by his wife and three children, Philip, Betsey and Nathan. From 1910 to 1915 he served as part-time assistant in my work on Drosophila. In the course of this work (MORGAN and BRIDGES 1913) certain exceptions turned up that BRIDGES began to study intensively. In 1913 he published briefly his results under the title of "Non-Disjunction of the Sex Chromosomes of Drosophila." A much more extended paper appeared in 1916 entitled "Non-Disjunction as Proof of the Chromosome Theory of Heredity." This work, offered as his doctoral dissertation at Columbia University, included not only genetic evidence but corresponding evidence from a study of the chromosomes that tallied with the genetic results. This paper went far towards convincing skeptics and conservatives that chromosomes are the bearers of genetic factors. It is true there was abundant evidence before 1916 showing that chromosome behavior furnishes an interpretation of heredity. It is today hard to believe that it was nearly ten years before this relation was generally accepted.

Sex-linked inheritance, as connecting genetic interpretation with known transmission of the sex chromosomes, had been established since 1910. The interpretation of crossing over, supported by less certain cytological evidence, had also been advanced in 1910, and covered what seemed to be exceptions to MENDEL's laws. Crossing over opened up a new field of research in inheritance, and for 30 years formed the material on which much of BRIDGES' work rested. He greatly improved the technique of locating the genes, and in the course of the following years he so thoroughly built up the genetic maps that these stand today as the most complete data we have of the location of the genes in the chromosomes.

During 1915–1916 BRIDGES held a University Fellowship at Columbia and from 1915 until his death he was associated with the Carnegie Institution of Washington first as assistant (1915–1919) and later (1919–1938) as staff member.

From 1916 to 1928 he was a member of the Carnegie group of investigation working at Columbia University, and from 1928 to 1938 at the California Institute of Technology. The annual grants from the Carnegie Institution of Washington contributed greatly towards the success of the genetic work on Drosophila.

In 1916 BRIDGES and I brought together the then known data on the mutants and linkage relations of the first or X chromosome of Drosophila, in 1919 those of the second chromosome were published, and in 1923 we

published the data which had accumulated on the third chromosome group of mutants. Later (1935) BRIDGES gave a complete account up to date of the fourth chromosome (Journal of Biology, Moscow, U.S.S.R.).

The first gynandromorph of Drosophila was discovered in 1910. From that time onward such exceptions were watched for and recorded. In time Bridges and I had a considerable number on hand which we described in a Carnegie Publication in 1919. Bridges reported (1939) that we had at that time studied "about 100 gynandromorphs and found that the maternal X was eliminated about as often as the paternal X. In experiments in which all flies were counted 40 gynandromorphs occurred in 88,000 flies." In addition to our explanation of elimination of one of the X chromosomes, during early development, a few other exceptions were found that called for a different explanation; namely, the presence of two separate nuclei and reduction products in the egg.

Later BRIDGES discussed a few individuals showing spots on the body which, from genetic evidence, were composed of haploid cells, and, in some of these mosaics, the regions included body parts that enabled one to diagnose their sex. The spots were clearly female in constitution, and this was surprising at the time since in other insects, where haploid individuals were known (bees, etc.), they were males. BRIDGES' finding in Drosophila was, however, consistent with the female sex formula: two X plus two sets of autosomes. In the spots the same balance is present, that is one X and one set of autosomes, so that the haploid somatic tissue is female.

During the years 1921–1925 BRIDGES made an extensive study of certain types of intersexes in Drosophila. He showed clearly by cytological analyses that they were due to chromosome aberrations of a type that had not been established previously for intersexes of other insects. The intersexes showed complex mixtures of male and female parts. In the first culture in which they were found (1920) there were 37 of them in addition to 9 regular males and 96 females. They were completely sterile but certain of their sisters, when bred, gave intersexes and were shown by genetic and cytological evidence to be triploid (3N) females. The breeding experiments with such females showed that a certain proportion of their mature eggs contained one full set of chromosomes, one X and one of each autosome, and, in addition, part or all of one extra set. Those mature eggs that contained a diploid set of chromosomes, would, if fertilized by a normal X-bearing sperm, again give 3N females, and if fertilized by a Y sperm would give intersexes. The formula for the intersexes is $3A+2X+Y$. They differ from standard females only in having an extra set of autosomes, and this fact, as BRIDGES pointed out, "proved that autosomes (A) are as much determiners of the normal sex differences as are the so-

called sex chromosomes. Autosomes turn the scale toward maleness." This idea of genic balance he had already developed in 1921 in connection with changes induced by loss or gain of a fourth chromosome. The theory was applied to the interpretation of certain other abnormal-appearing types of individuals, notably superfemales and supermales. A superfemale, he showed, arises when an individual has three X chromosomes and two autosomes. A supermale arises when one X and three sets of autosomes are present. Three types of females may exist in which the balance between X's and autosomes is the same as in the normal (2X+2A) female, namely, 3X+3A and 4X+4A, besides the normal. These conclusions of BRIDGES were not theoretical speculations, but in every case the interpretation rested on genetic evidence, and in triploids and diploids on a cytological demonstration of the presence in the individual in question of the constellation of the chromosomes that was postulated.

BRIDGES paid a great deal of attention to the problem of genic balance, pointing out that it is fundamental for an understanding not only of the balance concerning sex, but for all other characters as well. The fact is that today there is demonstrable evidence that there is not a single gene for femaleness and another for maleness but several, perhaps many, genes distributed through the chromosomes and affecting the development of certain characters in one way or in the opposite way. This conception in its broadest aspects has always been insisted on by the group working on the genetics of Drosophila, although it is also true that a change in a single gene often leads to striking changes in the individuals containing such a mutant gene. They argued that both the old and the new gene influence the end result, not by acting alone but by collaborating with other genes, and, in the last analysis, with all or most of the genes to different degrees. Each gene is thought of as a differential.

BRIDGES' early discovery (1917) that certain genetic data could be interpreted as due to deficiencies in the chromsome construction has led in recent years to a factual demonstration of such deficiencies. In some of his latest work (1937–1938) he made use of this discovery in the interpretation of overlapping deficiencies in analyzing the characteristics of certain mutant types. It would be hard to find in the history of genetic research a more convincing demonstration of the combination of factual evidence and masterly interpretation of it. As early as 1919 BRIDGES described "duplication" as a chromosomal aberration, and here, as in his other work, his conclusions rested not on vague hypotheses but on experimental proof. Much later he also reported the occurrence of "repeats" in the normal chromosome which will have to be seriously considered in future interpretations of certain types of genetic behavior.

The many interesting problems connected with losses (deficiencies)

and additions (duplications) of groups of genes present interesting problems in which genic balance is involved, and the possibility, that now exists, of detecting deficiencies and duplications in the salivary chromosomes and correlating the observations with the genetic location of mutant genes is well under way, and during the last two years of his life, was receiving BRIDGES' close attention.

In 1925 the data, that had been collected in the course of genetic experiments, was brought together under the title "The Genetics of Drosophila," by T. H. MORGAN, C. B. BRIDGES, A. H. STURTEVANT. Not until 1934, when the first number of Drosophila Information Service edited by BRIDGES and DEMEREC appeared, was a similar summary made. BRIDGES spent a tremendous amount of hard work in summarizing the data, particularly those of the stocks that the Carnegie group had built up at Pasadena, and the reports include also much unpublished data that BRIDGES himself had on hand. Eleven numbers of Drosophila Information Service have appeared, the latest in 1939.

In recent years BRIDGES spent much time in correlating the loci of the genetic maps with the bands of the salivary maps. He made an elaborate study of the salivary chromosomes, and more than doubled the previously known number of bands. His maps have become the standard ones for *Drosophila melanogaster*. As I have pointed out elsewhere, the identification of the salivary bands with the loci on the genetic map would not have been possible were it not that during the preceding twenty-three years the genetic (crossover) maps had been built up to a point where such comparisons have a real demonstrable basis. While many workers had contributed to bring the genetic maps to their status of 1933, it was BRIDGES in particular who had made a more detailed and critical study of the maps than had any other one of his contemporaries.

As a member of the Carnegie group each year's progress was reported in the Year Book of the Carnegie Institution of Washington. These twenty-three reports give in briefest summary the results that BRIDGES had obtained. Whether the elaborate data on which these reports rested, can ever be fully utilized is questionable; but BRIDGES accomplished so much other work, that these will not be needed to place him amongst the leading geneticists of his time.

T. H. MORGAN

CYTOLOGICAL AND DEVELOPMENTAL STUDIES OF HYBRIDS BETWEEN MEDICAGO SATIVA AND A DIPLOID FORM OF M. FALCATA

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THE two species, *Medicago sativa* and *M. falcata*, are commonly considered as varieties of one highly polymorphic species, since they intercross readily (WALDRON 1920, TYSDAL and WESTOVER 1937) and all intergradations between the two main types may be found. *M. falcata*, however, exists in both the diploid and tetraploid form (FRYER 1930), and it is only as the tetraploid that it crosses readily with the tetraploid *M. sativa*. Heretofore, no successful cross between the diploid *M. falcata* and *M. sativa* has been reported. The present paper describes two triploid hybrids formed when the diploid form of *M. falcata* was the male parent, and several tetraploid hybrids formed when it was the female parent in crosses with *M. sativa*.

Histological studies were made in an endeavor to find the origin of the tetraploid hybrids and an explanation for the frequent failure of development of the triploid hybrids. Conditions in the developing ovules following crosses between plants of different chromosome number in Medicago are similar to those in Avena (KIHARA and NISHIYAMA 1932), Triticum (WAKAKUWA 1934, BOYES and THOMPSON 1937) and Nicotiana (KOSTOFF 1930). Fertilization occurs, but apparently the relation between the nuclear and cellular divisions in the endosperm is upset, so that many wrinkled, inviable seeds are formed when the female has the lower chromosome number but fewer, plumper, more viable seeds when the female has the higher chromosome number. The causes of the failure and the reciprocal cross differences in Medicago seem to lie in the endosperm as in the plants previously studied, though Medicago seeds normally have no endosperm at maturity.

MATERIALS AND METHODS

A yellow-flowered variety of alfalfa of unknown origin was obtained through A. H. WRIGHT at the University of Wisconsin. R. A. BRINK found that it did not cross readily with *Medicago sativa*. The somatic chromosome number was shown to be 16 by D. C. COOPER. The variety is winter hardy and may originally have been introduced from Siberia. It has been identified as *M. falcata*, a determination confirmed by H. A. SENN, Assistant Dominion Botanist, Ottawa, Canada. The plants are closely similar to and cross freely with those described by FRYER (1930) as the diploid form of *M. falcata*.

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Plants of the tetraploid *Medicago sativa*, the diploid *M. falcata* and several other diploid species of Medicago were grown in the greenhouse during the winters of 1936, 1937, and 1938. Artificial illumination was used to force the plants into flower in April. A few plants, transplanted from the field in September, 1938, flowered in October. Emasculations were made by an electrically driven vacuum pump following the method described by KIRK (1930). Pollinations were made by applying pollen, collected on emery paper, mounted on a toothpick (BRINK 1934). The seeds matured in about thirty days and were then grown in a flat before the seedlings were transplanted to the field.

Root tips for somatic chromosome counts were collected directly into Karpechenko's modification of Navashin's fixing fluid. Bud material for the study of chromosome behavior during meiosis and pod material for the study of the development of the embryo and endosperm were immersed in Carnoy's solution (3:1) for about a minute before placing in the Karpechenko fixative. The material, after being allowed to fix for about 24 hours, was dehydrated, cleared in cedarwood oil, embedded and sectioned. Meiotic and root tip materials were stained by the iodine-crystal violet-picric acid method. The ovary material was stained with Heidenhain's iron alum haematoxylin. Some meiotic material collected in Carnoy's solution was studied in aceto-carmine. Pollen grains were examined in lacto-phenol-methylene blue.

DEVELOPMENT FOLLOWING RECIPROCAL CROSSES

Two triploid hybrids were obtained in 1937 by R. A. BRINK after crossing a particular strain of *Medicago sativa*, known as K40, by the diploid *M. falcata* (table 1). Although over 700 flowers of *M. sativa* were pollinated by *M. falcata* in 1938, no more triploid hybrids were obtained. Since the K40 strain was not used in 1938 it may be possible that different lines of *M. sativa* differ in their crossability (BACKHOUSE 1916, MANGELSDORF and REEVES 1931, KARPECHENKO 1937) with the diploid form of *M. falcata*. When *M. falcata* was used as the female no triploid hybrids were obtained in either 1937 or 1938, though the ovaries started to develop in practically every case. About 60 percent of the ovaries matured but they contained only small, shrunken, inviable seeds and a few viable seeds which proved to contain tetraploid hybrids. A study of the differences in the reciprocal crosses was made in order to understand more clearly the reasons for the results.

A summary of the observations of the embryos developing following the cross *M. falcata* ♀ × *M. sativa* ♂ is given in table 2. Pollen tubes are found at the base of the ovaries, and 50 percent of the ovules are developing in the material collected 32 hours after pollination. The stage of de-

velopment is usually a 2-cell proembryo accompanied by 8 or 16 free nuclei in the endosperm. Seven chromosome counts obtained at metaphase of the first nuclear division in the proembryo show $2n = 24$ (figures 1, 2, also 5, and 9), proving that cross fertilization of the diploid by the tetraploid takes place.

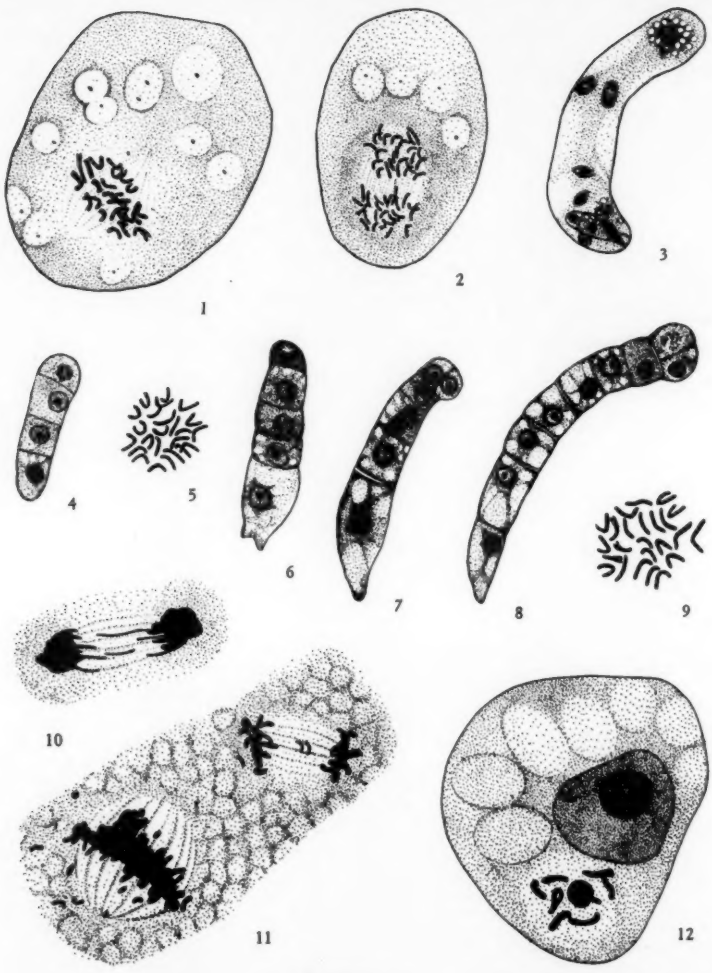
TABLE 1

Controlled greenhouse crosses of M. falcata (2n = 16) and M. sativa (2n = 32).

CROSS	YEAR	NO. FLOWERS POLLINATED	NO. PODS FORMED	PERCENT PODS FORMED	NO. SEEDS FORMED	NO. PLANTS GROWN	SOMATIC CHROMOSOME NUMBER
M. falcata (2n = 16) × *M. sativa* (2n = 32)	1937	—	—	—	11	5	16
						4	32
	1938	408	247	60.5	39	13	32
						8	—
M. sativa (2n = 32) × *M. falcata* (2n = 16)	1937	—	—	—	5	2	24
						3	32
	1938	774	30	3.9	28	12	32
						6	—

Embryos of *M. falcata* × *M. sativa* collected from two to six days after pollination show progressive stages of development (figures 3 to 8) as in *M. sativa* material (COOPER 1935). The rate of development, however, is considerably slower. In five-day material the proembryo varies from two to ten cells, having no more than four cells in the embryo proper in any case (table 2), whereas *M. sativa* material reaches the same stage of development in three days. The number of ovules developing decreases rapidly after six days but a few triploid embryos continue to develop and show traces of cotyledonary bulges at 18 days. Degeneration does not occur conspicuously at any one stage or in any particular part of the ovule, but all cells and nuclei become less deeply staining. Usually the inner integument becomes more meristematic in appearance and increases slightly in size and number of cells.

The endosperm develops rapidly at first, and 16 free nuclei are often present 32 hours after pollination, whereas only four or eight occur normally in *M. falcata*. Assuming that fertilization does not take place until 24–27 hours after pollination, as in *M. sativa* (COOPER 1935), it



FIGURES 1–12

Camera lucida drawings of embryo and endosperm development in *Medicago falcata* ♀ (n = 8) × *M. sativa* ♂ (n = 16), from paraffin serial sections. Magnification reduced one-half in reproduction to ×165 for figure 3, ×360 for figures 4, 6, 7, and 8, and ×1625 for the remaining figures.

FIGURE 1.—First nuclear division of the proembryo in metaphase, 2n = 24.

FIGURE 2.—First nuclear division of the proembryo in anaphase, 2n = 24.

FIGURE 3.—Embryo sac 2 days after pollination, two-cell proembryo, eight nuclei in division in the endosperm, chalazal nuclei more advanced.

FIGURE 4.—Four-cell proembryo at four days.

FIGURE 5.—2n = 24 chromosomes from apical cell of proembryo shown in figure 4.

TABLE 2

Embryo development following the cross Medicago falcata ♀ *(n=8)×M sativa* ♂ *(n=16).*

AGE AFTER POLLINATION	TOTAL OVULES	PERCENT OVULES DEVELOPING	TOTAL NUMBER OF CELLS											EMBRYOS OF KNOWN CHROMOSOME NUMBER	
			PROEMBRYO					EMBRYO PROPER							
			1	2	3	4	6	2	4	8	16	32	MANY	24	32
32 hours	521	50.1	61	200										7	—
2 days	196	27.0	19	34										3	—
3 days	195	33.8	7	38	15	9								2	—
4 days	189	38.6		11		51	12	8						2	—
5 days	334	38.0		14		30	30	30	2					8	—
6 days	44	47.7				2	1	2	4	9	2			1	—
8 days	72	13.9							1	2	4	3		—	—
11 days	72	10.0									2	5		—	—
18 days	99	10.0		1		1	2						6	3	1

must be concluded that the early development of the endosperm is rapid and exceeds that occurring in normal *M. falcata* material. The nuclear divisions in the endosperm, however, are not entirely regular (figure 10), and in older material occur less frequently. The nuclei attain various sizes as shown in figure 11, but are usually in the resting stage with irregular shape and numerous nucleoli. The endosperm may be completely resorbed before any sign of degeneration is evident in the embryo or integumentary cells.

One embryo in the 18-day material, though smaller than normal *M. falcata* embryos of similar age, is better developed than any *falcata* × *sativa* triploid embryo observed. It has prominent cotyledonary bulges, and 32 chromosomes were counted in several cells (table 2). The endosperm surrounding this tetraploid embryo differs conspicuously from that accompanying the triploid embryos in being cellular and normal in appearance. It is probable that this embryo would have become a tetraploid hybrid.

When *Medicago sativa* ♀ (n=16) is crossed with *M. falcata* (n=8), the flowers usually drop off in from three to five days (table 3). Thirty-two hours after pollination approximately 25 percent of the ovules are developing (figure 12), and the pollen tubes extend about half way to the

FIGURE 6.—Five-cell proembryo at five days.

FIGURE 7.—Seven-nucleate proembryo at four days.

FIGURE 8.—Nine-cell proembryo at five days.

FIGURE 9.—2n=24 chromosomes from the cell in division in the embryo shown in figure 8.

FIGURE 10.—Irregular nuclear division in the endosperm 30 hours after pollination.

FIGURE 11.—Large and small endosperm nuclei in division at six days.

FIGURE 12.—Delayed fertilization, showing the eight chromosomes of the male gamete in advance of the female nucleus when *M. sativa* ♀ (n=16)×*M. falcata* ♂ (n=8).

base of the ovaries. In two-day material fertilization is complete, and slightly more development has taken place in some ovules. Three-day material is no further developed, and many ovules have begun to degenerate. In the four- and five-day material the embryo sac is completely collapsed, and no sign of further development can be found, except in two cases which are probably the result of selfing.

TABLE 3

Embryo development following the cross Medicago sativa ♀ *(n=16)×M. falcata* ♂ *(n=8).*

HOURS AFTER POLLINATION	NUMBER OF FLOWERS	TOTAL NUMBER OVULES	NUMBER DEVELOPING OVULES	DEVELOPMENT	
				ZYGOTE CELLS	ENDOSPERM NUCLEI
32	23	219	53	1–2 proembryo	2– 4
48	25	212	94	1–3 proembryo	4–16
72	31	307	91	1–3 proembryo	4–16
96	20	214	1	2 embryo proper	16
120	79	870	1	16 embryo proper	16

Chromosome counts were obtained in six cases. The proembryo, in the four cases in which division was observed, has $2n = 24$. The endosperm in two cases has 40 chromosomes, only 8 of which are from the male parent. These counts prove that fertilization takes place. Development, however, is very slow; the nucellus does not disappear as in the normal *sativa* material but seems to increase in size and cytoplasmic density.

TRIPLOID HYBRIDS

The two triploid hybrids (figure 13) which were formed following the cross *M. sativa*×*M. falcata* (table 1) are intermediate between the parental types. The leaflets are longer and more serrate at the apex than are those of *M. sativa*. The flowers are variegated, and the color becomes more yellow as the flowers age. The two triploid hybrids differ considerably from each other. One is larger with long racemes and is more easily propagated vegetatively. The other is smaller with short racemes, darker flowers and leaflets which are more acute at the tip. The fertility and meiotic behavior of the two plants, however, is approximately the same.

The chromosome behavior in microsporogenesis is typical for autotriploids. Several scattered univalents are usually present during the first metaphase (figure 14), and sometimes eight bivalents and eight univalents can be recognized (figure 15). During the first anaphase five to eight univalents (figures 16 and 17) lie scattered in the spindle where they sometimes divide. The lagging chromosomes, not included in the two

nuclei at telophase, form micronuclei (figure 18). The second meiotic division is more regular (figure 19), and four microspores of approximately equal size are formed (figure 23). Univalents, divided during the first division, lag on the homoeotypic spindles (figure 20).

When all or nearly all of the univalents are included in one of the two nuclei during the first division, two large and two small nuclei are formed during the second division (figure 21). Micronuclei are frequently present after the second division (figure 22). One or two extra microspores, smaller than the other four, may be formed (figures 24 and 25), and 20 percent of one count of 224 microspores were of this extra type. The microspores are frequently of irregular size and shape (figure 26). Pollen grains from

TABLE 4

Medicago sativa-falcata derivatives obtained by backcrossing the triploid hybrid to the parental types.

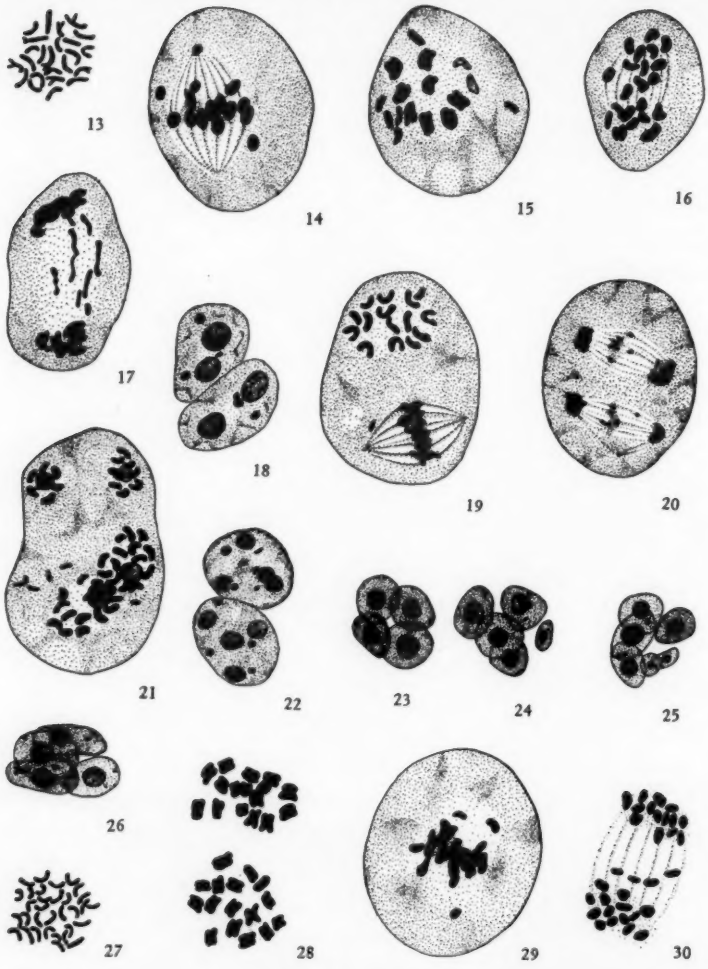
CROSS*	NUMBER INFLORESCENCES	TOTAL NUMBER FLOWERS	TOTAL PODS FORMED	PERCENT FLOWERS FORMING PODS	TOTAL SEEDS FORMED	SEEDS PER 100 OVULES	PLANTS OBTAINED	PERCENT GERMINATION
24×16	70	802	48	5.98	44	0.55	15	34
16×24	8	61	1	1.64	1	0.16	1	100
24×24	17	201	0	—	0	—	0	—
24 self	23	263	0	—	0	—	0	—
24×32	183	2187	157	7.18	80	0.36	31	39
32×24	38	308	18	5.84	26	0.84	11	46

* Parent plants are indicated by their somatic chromosome number. The female is given first.

the open flowers are greatly shrunken but about 20 percent contain deeply staining protoplasm, suggesting that the triploids would show some fertility.

The results of selfing and intercrossing the two triploid hybrids as well as outcrossing them both as male and female, to the diploid *falcata* and the tetraploid *sativa* types are summarized in table 4. No seeds were formed when the triploids were selfed or intercrossed, though many triploid flowers not included in the table were artificially self pollinated by rolling between thumb and fingers. In addition to the sterility arising from the irregular chromosome behavior, it is possible that self-incompatibility (BRINK and COOPER 1938) has increased the sterility of the triploids.

Sixteen plants were grown following hybridization between the triploid hybrid and the diploid *M. falcata*. One arose when the triploid was used as the male parent. It is of the *falcata* type but may not be the result of selfing since *M. falcata* plants are highly self-incompatible. Of the remaining plants, eight have 16, three have 17, and one has 18 chromosomes. The



FIGURES 13–30

Camera lucida drawings of triploid and tetraploid microsporogenesis, from aceto-carmine smears, and somatic chromosomes from paraffin sections. Magnification reduced one half in reproduction to ×740 for figures 18, 22, 23, 24, 25, and 26 and to ×1625 for the remaining figures.

FIGURE 13.—Triploid hybrid, root tip chromosomes, $2n = 24$.

FIGURE 14.—Triploid first metaphase, lateral, several univalents off plate.

FIGURE 15.—Triploid first metaphase, polar, $8_{II}+8_{I}$.

FIGURES 16, 17.—Triploid first anaphase, lateral, lagging of chromosomes.

FIGURE 18.—Triploid interphase, several micronuclei in each pollen mother cell.

FIGURE 19.—Triploid second metaphase, polar and lateral, regular.

chromosome behavior is usually regular. Most of the plants show a rather strong influence of the *sativa* type. The flower color varies from pale yellow to variegated with a predominance of yellow. The pods are usually more sickle shaped than in the original *falcata* type.

A considerable number of seeds were obtained when the triploid was crossed with *M. sativa*. Few plants, however, were obtained when the triploid was the male parent. They are of the *sativa* type and are probably the result of selfing or contamination of the *sativa* female parent. About thirty plants were obtained from seeds formed on the triploid plants following outcrossing with *M. sativa*. While variable, these plants, in general, conform to the *sativa* type. The flowers on nearly all of them, however, show some trace of yellow pigment and the pods are more loosely coiled than in *M. sativa*. Chromosome counts from sixteen of the plants range from 30 to 36 but are usually 32, in which case the chromosome behavior is regular.

The above results show that the female gametes of the triploid hybrids which have produced viable plants on outcrossing have 8 to 22 chromosomes. Apparently only those with 8 to 10 chromosomes are functional when the male parent is *M. falcata*, whereas those with 14 to 22 chromosomes are functional when the male parent is *M. sativa*. Evidently macrospores with a wide range in chromosome number are formed but few form viable gametes and their development depends partly on the chromosome number of the male gamete. The intermediate variable character, the high fertility, and the chromosome number of the *falcata* and *sativa* outcross derivatives of the triploid hybrids suggest that the *falcata* and the two sets of *sativa* chromosomes pair freely.

TETRAPLOID HYBRIDS

The cross, *Medicago falcata* ♀ (n = 8) × *M. sativa* ♂ (n = 16), gave seventeen plants (table 1) which, on cytological examination, were found to have 32 as the somatic chromosome number (figure 27). Explanations in terms of haploid or diploid androgenesis do not apply since the flowers are

FIGURE 20.—Triploid late second anaphase, with lagging chromosomes.
FIGURE 21.—Triploid late second anaphase, following an unequal distribution of univalents during the first division.
FIGURE 22.—Triploid pollen mother cells, after the second division, with micronuclei.
FIGURE 23.—Triploid, four typical microspores.
FIGURES 24, 25.—Triploid microspores, with one or two extra cells.
FIGURE 26.—Triploid microspores, four cells of irregular shape.
FIGURE 27.—Tetraploid hybrid, root tip chromosomes, 2n = 32.
FIGURE 28.—Tetraploid first anaphase, regular, 16+16.
FIGURE 29.—Tetraploid first metaphase, lateral, several univalents.
FIGURE 30.—Tetraploid second anaphase, lateral, four lagging chromosomes.

variegated, as would be expected in true hybrids. The plants are evidently tetraploid hybrids which have received twice the normal number of chromosomes from the diploid female parent. The flower color is closer to that of the diploid yellow-flowered parent while the size of the flowers and leaves is closer to that of the tetraploid parent. The 25 plants (including eight in which the chromosome number was not determined) are the result of 17 different crosses involving six different *sativa* plants and nine different diploid *falcata* plants. No more than one viable seed was obtained from any one flower pollinated so the increased chromosome number must have occurred in individual ovules.

The tetraploid hybrids when selfed formed only 0.1 seed, while on intercrossing different plants of this type 2.1 seeds were formed, and on outcrossing 320 tetraploid hybrid flowers to *M. sativa* 3.3 seeds were formed, on the average, for each flower pollinated. Evidently the tetraploid hybrids have a degree of self-incompatibility intermediate between that of the two parental types. The difference in seed set obtained on intercrossing and outcrossing the tetraploid hybrids may be due in part to chromosomal irregularities.

Cytological examination of the tetraploid hybrids usually shows a regular behavior at meiosis. In the first division 16 bivalents (figure 28) are commonly formed. Lateral views of first metaphase plates, however, frequently show several univalents (figure 29). The univalents often divide during the first division and are commonly seen lagging between the two main groups of chromosomes during the second division (figure 30). The irregularities may be sufficient to lower appreciably the viability of at least the male gametophyte.

The tetraploid hybrids are fertile and pairing is usually normal. Since an unreduced set of 16 chromosomes has been received from the diploid *falcata* parent, these chromosomes probably form eight bivalents by autosyndesis. It is necessary to assume, therefore, that two sets of eight chromosomes have also been received from the *sativa* parent and that they too may pair by autosyndesis. The chromosome behavior in the tetraploid hybrids suggests that the *falcata* and *sativa* chromosomes are homologous and interpair freely.

Other crosses in Medicago were attempted between *M. lupulina*, *M. platycarpa*, *M. ruthenica* and another diploid (n=8) unidentified species on the one hand, and the diploid *M. falcata* and the tetraploid *M. sativa* on the other. The crosses were entirely unsuccessful.

DISCUSSION AND CONCLUSIONS

The difference in the ease with which hybrid seeds are obtained following reciprocal crosses between *Medicago sativa* (n=16) and *M. falcata*

(n=8) is similar to that occurring in Avena (KIHARA and NISHIYAMA 1932) and Triticum (WAKAKUWA 1934, BOYES and THOMPSON 1937) although, unlike the cereals, Medicago seeds do not have endosperm at maturity. Fertilization occurs, but is somewhat delayed when the female has the higher chromosome number. When the female has the lower chromosome number the rate of nuclear division in the endosperm seems to be out of balance with the synthesis of protoplasm and the formation of cells. When the female has the higher chromosome number, on the other hand, the nuclear divisions in both the embryo and endosperm are slower but cellular divisions in the endosperm occur early in both Avena and Triticum and some viable seeds are produced.

The quantitative chromosome balance of the three tissues, embryo, endosperm, and parental tissue, has been stressed in connection with ovule abortion (GAIRDNER 1926, MÜNTZING 1930). It has already been shown, however, that the chromosome number relation of the embryo to the endosperm cannot be the chief cause of the abortion (MÜNTZING 1933). The present paper now suggests, since the divergence from the normal balance is greatest in the case of the tetraploid Medicago hybrid embryos, that the relation of the chromosome number of the embryo and endosperm to that of the mother tissue is not the primary cause of the failure of development.

The genomic and chromosomal balance within the endosperm is considered the main reason for the reciprocal cross differences by WATKINS (1927), WAKAKUWA (1930) and THOMPSON (1930 a and b). This seems improbable, since in Medicago the genomes are closely similar, and the homologous chromosomes interpair freely. There are certain gene differences, however, and one or more of these may influence endosperm development sufficiently to cause the differences in reciprocal crosses in Medicago. The importance of a single specific gene in the success of a species cross has been demonstrated (HOLLINGSHEAD 1929).

In Medicago the tetraploids develop, whereas the triploids usually fail. An immunity reaction (KOSTOFF 1930), against the triploids only, seems an unlikely explanation. According to EAST'S hypothesis (1935) the triploid hybrids should develop as readily on the diploid parent as do the tetraploids, since both carry the same chromosomes although in different proportions. This interpretation, therefore, is also inapplicable.

When the male has the higher chromosome number the balance seems to be shifted in favor of more rapid pollen tube growth and subsequent nuclear divisions. The rate of physiological activity and cell division in the endosperm seem correspondingly decreased. When the male has the lower chromosome number the nuclei of the embryo and endosperm are not stimulated (compare figure 12) to a sufficiently rapid rate of division

(KIHARA and NISHIYAMA 1932). The developing mother tissue utilizes the incoming food materials, causing the abortion of the ovule.

When an unreduced gamete functions in the diploid, however, the chromosome relation is proportional to that normally present in the parent plants and balanced rates of physiological activity occur. The primary reason for the differences arising in reciprocal crosses seems, therefore, to be the rate of physiological activity which is initiated in the embryo and endosperm at the time of fertilization, and this is dependent in large part on the chromosome number of the functioning gametes.

Hybrids having more chromosomes than normally expected have frequently been reported (BREMER 1923, CRANE and DARLINGTON 1927, GAIRDNER and DARLINGTON 1931, PETO 1934, SKALINSKA 1934, COLLINS and LONGLEY 1935). In each case, as in the tetraploid Medicago hybrids, it is assumed that due to the functioning of an unreduced female gamete the extra chromosomes have been received from the mother plant. Many examples have been reported in which fertilization has apparently been by an unreduced male gamete. Unreduced gametes are frequently formed by restitution in hybrids (LEDINGHAM and THOMPSON 1938).

Other possibilities of the origin of the tetraploid hybrids must be considered, since in Medicago it is not easy to prove that unreduced gametes are responsible. An integumentary cell extending into the embryo sac (COOPER 1935, Plate I, M, O) might have been fertilized, but all 643 developing embryos examined in this study arose in the normal micropylar position. Secondly, the eight female chromosomes might divide and form a restitution nucleus before the male chromosomes have divided. This could occur since the nuclei do not fuse completely before the first zygotic division, and one group may be a little ahead of the other in normal alfalfa material (COOPER 1935, Plate II, B–I). This explanation, however, seems improbable since in divisions, including seven in which accurate counts were made, the chromosomes were all at the same stage; and if restitutions occur some amphidiploids should form. Both these hypotheses explain how tetraploid embryos may be formed, but the endosperm remains the same and would probably cause the abortion of the tetraploid as well as the triploid embryos.

Triploids, and sometimes tetraploids, should arise in natural populations of the diploid *Medicago falcata* if unreduced gametes are formed. These probably occur in some plants (LARSEN and WESTERGAARD 1938) but the abnormal development of the endosperm, when the male and female gametes have different chromosome numbers, would prevent the occurrence of triploids in Medicago.

The diploid *Medicago falcata* may occur with the tetraploid *M. sativa* without intercrossing (KLINKOWSKI 1933). There are, however, no dis-

tinctive differences between the two species; and when triploid and tetraploid hybrids are formed, the homologous chromosomes interpair normally. The diploid form of *M. falcata* may approximate an original type of common alfalfa, and in it occasionally autotetraploids may still occur. *M. sativa* crosses freely with all tetraploid forms arising from *M. falcata*, giving a highly polymorphic group, with 32 somatic chromosomes, within which species differentiation is impossible. The diploid *M. falcata*, on the other hand, forms a homogeneous group which can be distinguished by chromosome number, size characters, the inability to cross with *M. sativa*, and by various gene differences, since certain *sativa* characters are unknown in the diploid *M. falcata*.

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SUMMARY

The diploid form of *Medicago falcata* does not cross readily with the common tetraploid *M. sativa*. When the former is the female, fertilization occurs, and development of the embryo proceeds slowly for about two weeks. The ovary continues to develop and forms a mature pod even though the ovules abort. When *M. sativa* is the female parent in the cross, fertilization is delayed and development of the endosperm and embryo usually stops after the second day.

The difference in seed development in reciprocal crosses seems to depend on the rate of physiological activity and cell division initiated in the endosperm at the time of fertilization. The chromosome number of the male and female nuclei is of considerable importance in this connection. There is some evidence that if the development of the endosperm and embryo is too slow some part of the ovule or ovary develops at their expense until abortion occurs.

Only two triploid hybrids were formed when *M. sativa* was the female parent, although the cross was repeated many times. Usually eight bivalents and eight univalents were present at first metaphase but some female gametes were viable in outcrosses to the parental types. The derivative plants are highly variable but the flower and pod characters and the chromosome number tend to be close to those of the recurrent parent.

When the cross was made in the other direction and the diploid *M. falcata* was the female parent only a few tetraploid hybrids were obtained. The flower color and pod shape are closer to the *falcata* type than in the

triploid hybrids for in the tetraploids twice the usual inheritance of the diploid *M. falcata* has been received. The fertility of the tetraploid hybrids is high, 16 bivalents usually forming, and the next generation is highly variable.

Homologous chromosomes of *M. falcata* and *M. sativa* interpair freely and there is no criterion by which the forms can be separated into distinct species. The diploid form of *M. falcata* does not cross readily with *M sativa* due to faulty development after fertilization.

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SOMATIC MOSAICS IN THE DOMESTIC PIGEON

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MOSAIC or chimeric effects not of artificial origin may conveniently be divided into two classes, those which are frequent, and customarily associated with special genotypes, and those which are rare and unpredictable. Variegation and eversporting are examples of the former class. In the pigeon a condition known as "flecking" is of this sort. The other class includes gynandromorphs and chimeras generally.

These phenomena have received little attention in the pigeon. We shall present a review of the known facts, together with new cases which have come under our observation, and suggestions as to the possible causes involved.

FLECKING ASSOCIATED WITH SEX-LINKED FACTORS

In the wild type or "blue, black-barred" pigeon (*Columba livia*) no flecking has been observed. Flecking is found associated with three sex-linked color factors, which, as indicated later, are probably all alleles. Each of these is dominant to its wild-type allele, and each is responsible primarily for a particular kind of "bleaching" effect, that is, the plumage is lighter in color than that of the wild type. The apparent mutation, inactivation, or loss of such a factor in areas of irregular size and distribution in the feathers produces the flecking.

The most common of these factors is the "dominant red" of COLE and KELLEY (1919), or, as we shall call it, more specifically, "ash-red" (figure 1). HAWKINS (1931) demonstrated that the factor for sex-linked recessive "chocolate-brown" plumage color is a third allele at the ash-red locus. He symbolized these alleles, in descending order of dominance, B^A (ash-red), B (wild type), and b (chocolate-brown). HAWKINS reviewed the literature on flecking and reported new observations of his own. He found flecks only in heterozygous males, and suggested that flecking is merely a consequence of heterozygosity. Thus, males of the constitution B^AB showed only flecks of B phenotype, while males of the constitution B^Ab showed only flecks of the b phenotype; females, since they possess but one sex chromosome, cannot be heterozygous in the strict use of the term.

However, STEELE (1926) had definitely stated that he had observed "dunnish" flecks in dominant red females. We also have found flecking in many such females, and the flecks are invariably of the b type. HAWKINS'

[1] Papers from the Department of Genetics, Agricultural Experiment Station, No. 246.

suggestion that flecking is due to heterozygosity as such is therefore inadequate.

The sex difference in flecking was vaguely recognized for a long time by breeders (Cf. DARWIN, 1900, Vol. I, Chap. 5, p. 167), but the first clear outline was given by STEELE (1926). He stated that males more often exhibit flecking than do females; that flecked males generally show more flecks than do flecked females; and that flecks in females are not as dark in color as those in males. HAWKINS (1931) attempted to find whether an

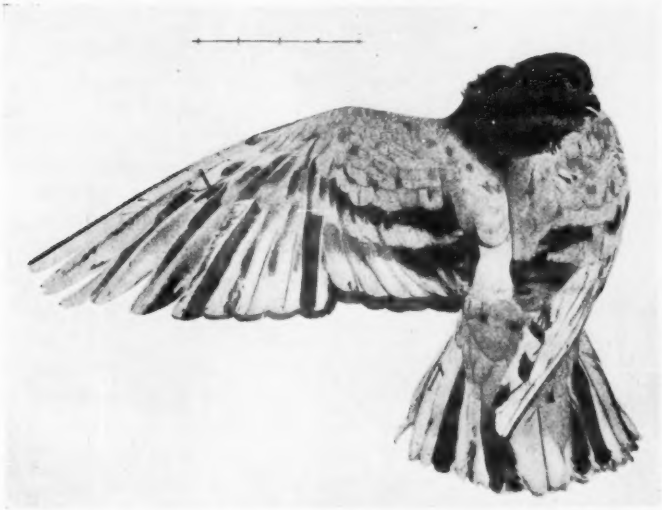

FIGURE 1.—Male D325Z, age 8½ years, showing large amount of black flecking. Genotype: $B^A B$ (ash-red).

endocrine differential is involved by castrating flecked males and observing subsequent feather regeneration. No change in fleck color or in general incidence of flecking was found.

Age has an important influence on flecking. The sex differences are already apparent in the juvenile plumage, but the flecking tends to increase in amount with each moult, so that, in old males particularly, it is extremely marked (figure 1).

The second most common factor associated with flecking is "almond," symbolized *St* by WRIEDT and CHRISTIE (1925). The color is basically yellowish-ashy to white, depending on associated factors. This factor is characteristic of the almond Tumbler, Oriental Roller, and Magnani Modena breeds. Flecking is abundant in both sexes, but as GHIGI (1908)

has shown, there is a marked sex-difference in quantity, as with ash-red. Unlike ash-red, the flecks in almond females are not chocolate in color, but show black (wild type) pigment. The flecking increases with each moult, as in the case of ash-red; old males may become more black than almond in appearance.

The third factor associated with flecking is "faded," *Of*, (HOLLANDER 1938). It has since been proved to be sex-linked. The general effect of this factor is much less obvious than almond or ash-red; the plumage is only slightly bleached. Flecking in faded birds is about the same in amount as that in heterozygous young ash-red males. It differs from ash-red and almond in that no sex difference or age difference has been apparent, but as in almond, the females have black pigmented flecks.

The relationships of all these three factors to one another are not yet fully determined but allelism is strongly indicated. Linkage tests of almond with another sex-linked factor, the recessive "dilution," *d*, were made by WRIEDT and CHRISTIE (1925); crossing over was around 50 percent. The amount of crossing over between ash-red and dilution was also very high in tests made by COLE and KELLEY (1919) and subsequent investigators. Since almond and ash-red both appear to lie at a distance from dilution, they probably are close neighbors of one another, if not actually allelic. FELDMAN (unpublished)[2] made a test of this possibility. Males heterozygous for ash-red and almond were produced; these resembled almond except that the flecking was of the ash-red color. Preliminary breeding tests with these males gave no crossing over.

We have obtained the male heterozygote of almond and chocolate. In this case a most unexpected result appeared: the flecks are chiefly chocolate, but a few are wild type. No linkage tests have yet been attempted. Faded has not yet been tested with almond or with chocolate, but it should be noted that FELDMAN obtained faded originally in the son of an almond ("Parlor Tumbler") female. By the rules of sex-linked inheritance, this son should have been almond; the best explanation so far advanced is that almond gave rise to faded by mutation. In preliminary linkage tests with dilution, faded has given a high rate of crossing over, while with ash-red it has given only non-crossovers. Males heterozygous for ash-red and faded resemble ash-red in general but their flecks are of the faded coloration.

In view of the above indications that almond and faded are situated at the B^A locus, we shall for purposes of discussion assume that all are alleles, and refer to them as the *B* series.

[2] The work referred to here was done by DR. H. W. FELDMAN at the University of Michigan He has generously given us valuable birds of the almond and faded types, as well as access to his records.

FLECKING ASSOCIATED WITH AN AUTOSOMAL FACTOR

Only one autosomal factor has been found accompanied by flecking. This factor is "grizzle" (*G*), also dominant to the wild type, and responsible for a whitening of the plumage. In a few heterozygotes we have observed large portions of feathers clearly lacking the grizzle factor, and therefore quite comparable to flecking associated with the sex-linked factors. It is difficult however to be certain that small areas lack grizzle. No further study of the flecking with this factor has been undertaken.

MUTATION IN THE SEX-LINKED B SERIES

This series may be symbolized provisionally in descending order of dominance, B^{St} (almond) > B^{A} (ash-red) > B^{Of} (faded) > B (wild type) > b (chocolate). The general effect of the genes dominant to wild type is to reduce the black pigmentation gradually to light gray or white in some areas and to red or yellow in others. These light colors serve as a contrasting background for dark flecks of a more recessive color. On the hypothesis that the dominant gene is completely lost or inactivated in the area of the fleck, the color of the fleck should be governed by the remaining allele. Thus if the gene B^{A} were eliminated in the heterozygote B^{A} B the fleck pigmentation should be wild type, while in the heterozygote B^{A} b the fleck pigmentation should be chocolate. In females of any of the three dominant types chocolate flecks (the "residual" condition) would be expected on the assumption that b is the lowest possible allele at this locus, but such is not the case for almond or faded. Furthermore, the male almond-chocolate heterozygote, B^{St} b, has not only flecks of the expected chocolate pigmentation but also some of wild type. The conclusion seems unavoidable that there is not actual loss or complete inactivation of the dominant allele in all cases. This also precludes an explanation based on the loss of an entire chromosome and further evidence against such loss is found in males heterozygous for both the ash-red and the dilution loci of the sex chromosome: B^{A} D/B d. The flecks in such males were always black. If the whole chromosome containing B^{A} were lost also in some cases, the flecks should then show the dilution phenotype, provided that this factor can produce its effect autonomously in development. That it can so act is indicated later in connection with chimeras.

A more plausible genetic basis for the flecking seems to be offered by the hypothesis of somatic mutation of labile genes. Such genes have been shown to exist in a number of species of animals and plants. In this case it is only necessary to assume that the dominant genes tend to mutate to alleles lower in the series, but not always to the same one even in a single bird. Presumably these mutations occur in homozygotes equally as often as in heterozygotes, but no visible result would be expected unless both

genes in the cell mutated. Similarly, in males heterozygous for two of the dominants, mutation of the lower allele would never be observable unless by coincidence the higher gene mutated also. There is no evidence that either the wild type or chocolate alleles are at all labile; apparently mutation is a characteristic of the genes dominant to wild type and the higher the allele in the series, the more labile it is. Since almond heterozygotes show such a large amount of flecking, we would not be surprised if almond homozygotes should show a few flecks; the mutation rate seems so high that both genes might well occasionally mutate in the same cell. However, no observations on mature male almond homozygotes have yet been made.

In table 1 is summarized present knowledge of the *B* series genotypes and phenotypes, with notes on the flecking.

FLECKING IN OTHER BIRDS

The pigeon is not alone among birds in exhibiting flecking. Essentially the same phenomenon, under names such as "fault feathering" or "exceptional feathers," is found in the "blue" domestic duck, the slate turkey, and several sorts of chickens, such as Barred Plymouth Rocks, Andalusians, and dominant whites. In certain of these forms it has received considerable attention both from breeders and from investigators, and has been recognized as a special phase of the problem of coloration because of the high degree of irregularity. In all these species, as in the pigeon, the genetic factors involved are more or less dominant to the wild type; furthermore, the flecking does not occur in homozygotes, except in the white Andalusian fowl. The Andalusian, seeming exceptional, requires special discussion. SEREBROVKSY (1926) states that flecking in the heterozygous ("blue") Andalusians is always black (wild type allele), while it is "blue" in homozygous whites, and much more striking than in heterozygotes. He explains these facts by the assumption that "loss" of one allele occurs. The homozygote having two labile alleles, blue flecking should be twice as abundant as in heterozygotes. We have examined a number of Andalusians, and find SEREBROVSKY'S treatment satisfactory. We may add that within large blue flecks (in homozygotes) black flecks are occasionally to be found. These would indicate "loss" of both alleles. The reason for the peculiar status of the Andalusians seems simply the fact that the factor involved is only partially dominant to the wild type allele, the heterozygous condition being phenotypically distinct.

The Andalusian factor and dominant white in fowls are not sex-linked, and apparently sex-linkage is not involved in the duck or turkey. But most of the investigation of the flecking has centered around two independent dominant sex-linked color factors, "bar" and "silver," in the chicken. SEREBROVKSY (1926) and HERTWIG and RITTERSHAUS (1929)

TABLE 1

Genotypes and Phenotypes of the B Series.

GENOTYPE	BASIC COLOR	FLECKS	EXPECTATION ON BASIS OF LOSS
		Almond Genotypes	
Males			
$B^{St}\ B^{St}$	*	*	Creamy; no flecks
$B^{St}\ B^{A}$	Creamy	Ash-red	As found
$B^{St}\ B^{0f}$	*	*	Creamy; faded flecks
$B^{St}\ B$	Creamy	Black	As found
$B^{St}\ b$	Creamy	Chocolate and black	Only chocolate flecks
Females			
$B^{St}-$	Creamy	Black	Chocolate flecks
		Ash-red Series	
Males			
$B^{A}\ B^{A}$	Ash-red	None	As found
$B^{A}\ B^{0f}$	Ash-red	Faded	As found
$B^{A}\ B$	Ash-red	Black	As found
$B^{A}\ b$	Ash-red	Chocolate	As found
Females			
$B^{A}-$	Ash-red	Chocolate	As found
		Faded Series	
Males			
$B^{0f}\ B^{0f}$	*	*	Faded, no flecks
$B^{0f}\ B$	Faded	Black	As found
$B^{0f}\ b$	*	*	Faded; chocolate flecks
Females			
B^{0f}	Faded	Black	Faded; chocolate flecks
		Black Series	
Males			
$B\ B$	Black	None	No flecks
$B\ b$	Black	None	Chocolate flecks if mutation occurred
Females			
$B-$	Black	None	Chocolate flecks if mutation occurred
		Chocolate	
Males			
$b\ b$	Chocolate	None	As found
Females			
$b-$	Chocolate	None	As found

* Not studied or insufficient information.

observed the frequencies of each kind of fleck in Barred Plymouth Rocks and crosses possessing both these factors, and in each sex. SEREBROVSKY concluded that segregation of whole sex chromosomes, with or without crossing over, would adequately explain the facts, while the latter authors decided that only part of the chromosome—usually the part containing

the bar factor—was lost. In either case, a genetic change in the soma appeared to account for the exceptional feathers.

HERTWIG and RITTERSHAUS gave as further evidence of a genetic change that, after plucking, the fault feathers are replaced by new ones of the same type. JUHN (1933) has demonstrated however that this result is not regularly found. She concludes that her findings "do not support the genetic interpretations advanced," which, she adds, "are untenable from an embryological point of view," though she does not specify in what way. Her explanation of the exceptional feathers is that a physiological threshold allows the recessive factors in the birds' make-up to function at times, depending on "the interaction of the genetic factors . . . with variable morphogenetic factors such as rate of growth . . . " In other words, a physiological control of the action of these genes is postulated, the dominant being expressed under certain local internal conditions, and suppressed under others.

MONTALENTI (1934) has also investigated feather succession in Barred Plymouth Rocks. He found that follicles from which "abnormal" feathers are plucked always regenerated abnormal feathers, though in most cases the successive feathers differed considerably. If the feathers were completely abnormal (black, in these chickens) they were generally followed by new ones of exactly the same sort. MONTALENTI sums up his observations and deductions as follows: "It appears . . . that the *range of action of the genes for the barring* in mosaic feathers may vary considerably in successive generations. Sometimes the barring does not appear at all in some of them, although this factor is potentially present in the follicles concerned, as it is proved by its manifestation in successive generations of feathers." (Italics ours.)

THE DEVELOPMENTAL BASIS OF FLECKING

We have concluded so far, on the basis of fleck coloration, that flecking in pigeons is the result of genetic change in the soma, and that this change is probably mutational in nature. We must next examine the facts of feather development to determine whether this conclusion can be accepted.

We have observed successive feathers from a large number of follicles in different specimens of the *B* series. As in chickens, there is a great deal of variability in the successive feathers; nevertheless, where the fleck is so large as to extend from the tip to the base of the feather, and especially if an entire vane or more is involved, repetition in successive feathers is obvious (figure 2). The smaller the flecks the less tendency there is to repeat in detail, but the same absolute variability would be expected to produce more apparent alteration of the small flecks than of the large

ones. At any rate, we may conclude that in the case of the great flecks, a relatively permanent differentiation exists in the feather primordium with respect to potentialities for color production.

If we interpret LILLIE and JUHN's latest (1938) contribution correctly, they now consider growth of the feather to be mainly axial, rather than concrescent as earlier assumed. Any genetic difference existing in the "collar," such as might occur from mutation, should therefore result in a

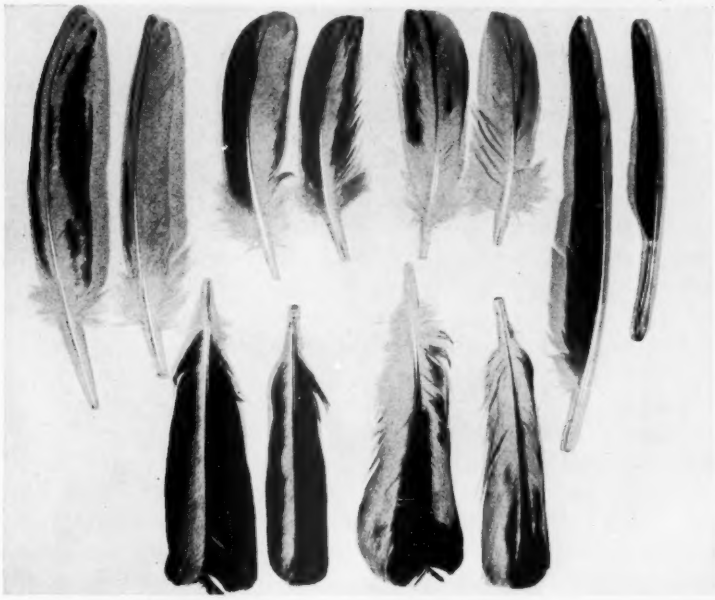

FIGURE 2.—Pairs of successive feathers from six follicles from aged flecked male, 2515E (genotype B^A B). In each pair, the younger feather is to the right of the older. Above, wing feathers; below, tail feathers. The first feathers were all pulled the same day, and the successors a month later. Note that the more outstanding fleck areas (black) tend to be repeated, with fair fidelity in most cases, and that the fleck areas in general run longitudinally.

corresponding longitudinal streak in the completed feather. This condition is often approximated by the larger flecks, and the structural difference shown in figure 15 gives striking evidence of definite axial growth. The small flecks appear as irregular islands. The irregularities in shape and size, as well as in replacement, may be accounted for by one or more of several processes: 1. New mutations which would affect very small areas may be occurring intermittently in the active part of the collar. 2. Irregularities of outline and lack of continuity, producing flecks rather than continuous streaks, may result from uneven relative growth of different

derivative cells in the collar. 3. As DANFORTH (1939) has emphasized, the early precursors of the pigment cells are apparently capable of migration; furthermore, these specialized cells are large and have long branching processes which deposit pigment some distance from the original position of the cell (GREITE 1934).

That physiological differences and changes may have an influence on the incidence of mutation and possibly on the relative growth of the mutated portions is as yet undeniable. Sex and age differences in the quantity of flecking, as described in connection with ash-red and almond, point to some measure of metabolic control. Subsidiary genetic factors, subject to selection, also may have an influence on the degree of flecking, as is shown by differences in specimens physiologically comparable.

CHIMERAS

Gynandromorphism is a well-known type of chimera in domestic fowls, according to CREW and MUNRO (1938), but no case has been reported in pigeons. The lack may be due to the difficulty in recognizing sexually abnormal pigeons; external differences between male and female are so slight that a gynandromorph might easily be overlooked.

Chimeras of feather color and structure have, however, occasionally been observed. The flecking and chimera types have much in common but chimeras differ from the flecking type in being relatively rare and unpredictable in occurrence, and in having larger areas affected, including whole groups of feathers, and even considerable portions of the body. For convenience, and without any necessary implication as to cause, these will be referred to as the "mutant areas." They are, in general, though not always, similar to flecking in being attributable to some sort of loss of the dominant allele in a heterozygote. To account for the large areas involved, it must be assumed that the "mutation" occurred earlier in ontogeny than is the case with flecking, possibly in some cases even as early as the first cleavage division.

For convenience of treatment, the pigeon chimeras which have come to our attention (26 in all) are classified below according to the principal factors involved.

1. *Chimeras involving ash-red* (B^A).

LYELL (1877, p. 48) states that he once bred a "mealy with black shoulders." It is clear from his other descriptions that by "mealy" LYELL refers to the ash-red condition, while the mutant area is of the *B* phenotype. He did not give sex or pedigree of the specimen.

Two somewhat similar male mosaics have been studied at this laboratory. The first, male 2067B, was a crossbred Tumbler produced by an

ash-red male and a black female. Most of the plumage of this specimen was typically ash-red with black flecks, but on the crop, back of the head, and inter-scapular region were large patches of black feathers. These patches remained during the six years of the bird's life. A progeny test indicated that his constitution was B^AB, as was to be expected. The second mosaic, 2411A, exhibited much more extensive B areas (figure 3).

FIGURE 3.—Diagrams of male chimera 2411A. In these and following diagrams, black represents the dominant allele, stippling represents the recessive allele, and white represents white-spotting. Dominant allele here, B^A (ash-red); recessive allele, B (blue checker).

This bird was a Homing pigeon obtained from a Milwaukee, Wisconsin, breeder; the parents were said to be an ash-red male and a wild-type-colored female. The mosaic was tested with a wild type female; of the five offspring obtained, two were typical ash-red, and three showed wild-type pigmentation. Thus the gonads were, at least in part, of the constitution B^AB.

LEVI and HOLLANDER (1939) report two additional cases, with illustrations. Both were B^AB males, by pedigree and, in the one case tested, by progeny test. Both showed a large amount of B plumage, and both possessed white-spotting, extensive in one. A most unusual feature of one of these mosaics was the change, in the transition from juvenile to adult plumage, of a large part of the B plumage in the tail back to the proper color, ash-red. Such reversion has not been observed in any other chimera.

A male bird (E284E) of genotype B^AB had relatively few and small flecks in its juvenal plumage. Now, in its first adult plumage, it has abundant flecks and by the time it is several years old it will presumably be fully as flecked as D325Z, shown in figure 1. In addition, E284E has on the left side of its head a black patch which extends from near the base of the beak, beneath the eye to the occiput (figure 4). The total area of this patch is less than might occur on a single tail feather; it differs from the latter, however, in that it involves a good many contiguous feathers instead of a single one, and its extent has not increased with the molt.

This specimen may accordingly be included as a chimera.

In the above six cases, the mosaicism can be accounted for by the hypothesis that the B^A factor, in heterozygotes, has mutated to B (or b) or become lost or otherwise inactivated, as in the case of flecking, but at some time early in ontogeny.

A more complicated problem is presented by male 2721E, bred at this laboratory. In addition to the sex-linked ash-red factor, an autosomal feather pattern factor is involved. The bird's father was a dun of the T

FIGURE 4.—Young ash-red male E284E (genotype B^A B) showing black flecks on individual feathers and a mutated patch of black feathers on the head. The latter presumably represent a single early mutation, making this bird, according to definition, a chimera.

pattern, genotypically $B\ d/B\ d;\ C^T/C$. The mother was a typical ash-red, of the constitution $B^A\ D/-;\ c/c$. The mosaic is ash-red in the checker pattern, with flecking, and is therefore genotypically $B\ ?/B^A\ D;\ C/c$. On the right wing there is a large patch of brownish feathers (enclosed by a black line, figure 5) whose color we have not been able to identify with certainty. The feathers of this area have a few flecks, which are black as in other parts of plumage. Furthermore, the feathers appear to be of the T pattern, rather than checker. Possibly the brownish color arose by recessive mutation of B^A to some new allele in the series, but a simultaneous mutation of C to C^T in another chromosome seems highly improbable. No very satisfactory explanation of this mosaic has been deduced. Polyspermy might perhaps be invoked but this would involve other complications. No breeding tests were made.

2. *Chimeras involving chocolate* (*b*).

A male mosaic showing irregular areas of *B* and *b* plumage coloration, together with a good deal of white spotting, is reported and figured by LEVI and HOLLANDER (1939), in the King breed. By pedigree, the bird's genotype was *Bb*. This case would be most simply explained by the loss of *B* in certain areas. This is the only case we have of the mutation of *B*.

FIGURE 5.—Right wing from male chimera 2721E. The island of unusual brownish feathers is outlined by a black ink line. The surrounding feathers are ash-red (B^A *B*) with a few black flecks; a single black fleck also occurs in a lesser secondary covert in the island.

3. *Chimeras involving dilution* (*d*)

Two mosaics of dilution and its normal allele are known, both in the Carneau breed. KEESLING (1924) described a specimen whose father was yellow (dilution with autosomal recessive red: *dd ee*) and whose mother was red (*D- ee*). It was "a cock of beautiful type and good size; has yellow head with small red spots, red breast and neck, yellow wings and back, red wing flights and tail. The markings are sharply defined." From this description it seems that a high degree of symmetry existed. The constitution of the bird is, by pedigree, *Dd*, so that the appearance of *d* areas is unexpected, but may be accounted for by mutation of *D* to *d*.

A quite similar case, though not symmetrically marked, was observed by one of us (W. F. H.) at the Middleton Squab Farm of Norristown, Pa. This bird also was a male, apparently breeding normally. There was a

moderate amount of white spotting in the plumage. Nothing further is known about this bird.

4. *Chimeras involving grizzle* (*G*)

A single grizzle mosaic female crossbred Homer-Carneau, *Gg* by pedigree, is reported by LEVI and HOLLANDER (1939). Most of the bird is grizzle but the factor appears to be lacking on most or all of the left wing. There was a moderate amount of white spotting.

5. *Chimeras involving recessive red* (*e*)

a. *With recessive mutant areas.* METZELAAR (1926, p. 34) mentioned a recessive red mosaic. In correspondence of January 24, 1925, he described it in detail: "Crossing a pure recessive red Carneau with a brown silver King, a female young was obtained which shows both parental colors in a piebald form. The flights are pure red, so is the neck; the rest of the body is brown-barred but for a few flecks in the areas with clumped pigment. These flecks are red; not clumped but spread pigment. They are irregular islands of spread pigment within the clumped brown region." The brown in this mating is sex-linked chocolate, and is to be expected in female offspring. The red, however, is unexpected, but may be accounted for by assuming mutation of *E* to *e* in a bird of the composition *b*/-; *E*/*e*. Here again is a case with (apparently) a fair symmetry. The bird has been mounted, and is at present in the Museum of Zoology at the University of Michigan.

Two other mosaics involving recessive red were given us in 1934 by DR. H. W. FELDMAN from the University of Michigan colony. These were male sibs, 2708.1 and 2708.2, F_2's from recessive red grizzle white-spotted Tippler × black-laced Blondinette. Both were black with some grizzling and white-spotting apparent; in one, scattered patches of red feathers occur over the neck, crop, and scapular regions; in the other, only a few scapulars are red. Although neither specimen was progeny-tested, their genotype was probably *Ee*, and the red areas may be accounted for simply by loss of the normal allele.

Two similar cases have appeared at this laboratory. A female, 1158H, whose skin in juvenile plumage was preserved, closely resembles the above males but no entirely red feathers are present; instead, there are large red segments of the interscapular feathers and elsewhere. She also had the typical juvenile reddish edging on most other feathers. She was heterozygous for *e*, as the father was recessive red and the mother black. The remaining case is a male, 2688H (figure 6), which has a rather large area of recessive red color, and also a moderate degree of white spotting. No breeding tests were made, but as both parents were heterozygous for *e*,

this specimen in all probability is also. Here again the appearance of red may be attributed to loss of the normal allele.

FIGURE 6.—Diagrams of male chimera 2688H. Black areas represent wild type allele (blue checker); stippled areas recessive red.

b. *With dominant feathers.* HORLACHER (1930, p. 341) mentions four recessive red specimens with one or more black feathers. Here we have

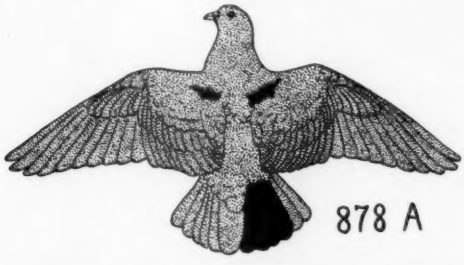

FIGURE 7.—Diagram of male chimera 878A. Stippling, recessive red; black, wild type allele (black).

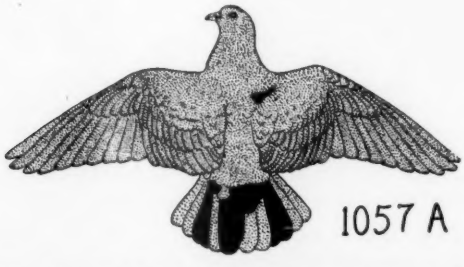

FIGURE 8.—Diagram of female chimera 1057A. Key same as in figure 7.

what appears to be a different sort of chimera from those treated above. Further information is available for two of these. One was a male, 1875C. Both parents were recessive red, and the first description of the specimen

(six years before HORLACHER's description) made no mention of any black feathers; HORLACHER found a single black wing covert in this bird. The other mosaic, 1057A, was a female (figure 8). This specimen has been

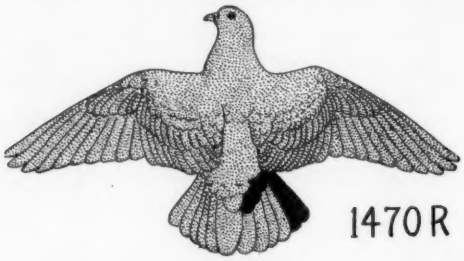

FIGURE 9.—Diagram of female chimera 1470R. Key same as in figure 7.

preserved; there are black feathers in the interscapular region and in the tail. No progeny test was made.

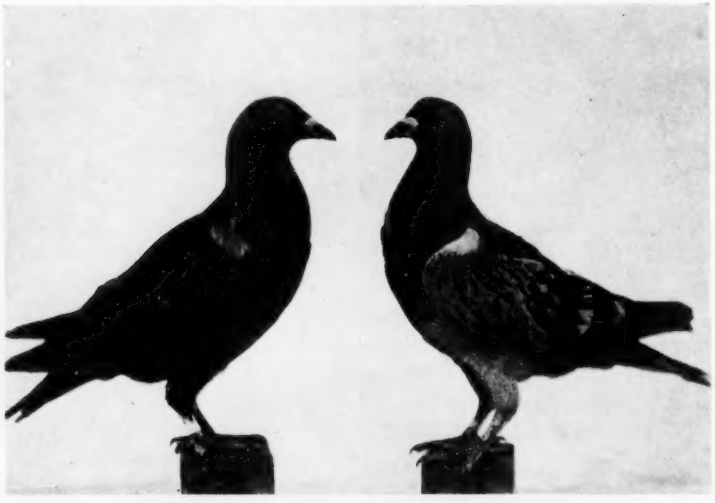

FIGURE 10.—Right and left aspects of "half-sider" *S* mosaic. Right side spread black, *S;* left side blue checker, *S*.

Two additional examples very similar to 1057A have been produced in the Wisconsin colony. One was a male, 878A (figure 7), and the other a female, 1470R (figure 9). These three mosaics are somewhat related to one another, the females being first cousins. Furthermore, a paternal uncle of the females, 925B, was described as recessive red with two black

coverts in the right wing. The parentage of all the above individuals was *Ee* male×*ee* female. It is therefore not possible to be certain of the genotype of the mosaics except by breeding test. The male 878A, mated with a recessive red, produced a total of three young, all typical *ee*. The female 1470R, in several matings with *ee* males, including one of her sons, produced 35 offspring, all typical *ee*, with no tendency to mosaicism. Apparently the genotype of these mosaics is therefore *ee*.

The only explanation which seems likely for this sort of mosaic is dominant mutation, but polyspermy could possibly, though very doubtfully, be invoked.

6. *Chimeras involving S ("spread black")*

A male Homing pigeon (sex?) black on the right side and blue checker on the left (figures 10 and 11) is now at the Whitman laboratory of the University of Chicago. It was bred by MR. CALDWELL MARTIN of Denver,

FIGURE 11.—Ventral aspect of "half-sider" *S* mosaic.

Colorado, and sent to DR. L. V. DOMM through the interest of MR. W. VAN RIPER of Denver. Apparently, the *S* factor, which produces "spread" blackness of plumage, is involved; both parents were black, but in another mating the sire proved heterozygous. It may be assumed that the initial constitution of the mosaic was *Ss*. Near the median division line some feathers exhibit both black and blue in longitudinal stripes (figure 12); this is apparently the result of genetically mixed tissue in the same feather follicle, since new feathers from these follicles have been very similar to the previous ones.

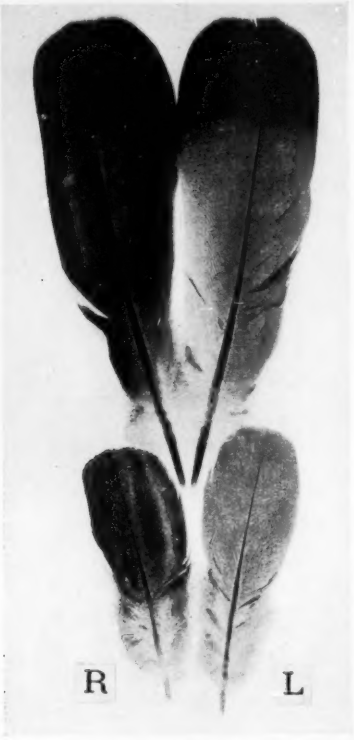

FIGURE 12.—Rectrices and dorsal major tail coverts from right and left sides of midline of "half-sider" *S* mosaic. Note the smoothly demarcated stripes on the feathers from the right side.

At the Wisconsin colony a more complicated mosaic involving *S* appeared. This specimen, D935K (figure 13), was killed while yet in juvenile plumage and before its interest had been fully recognized.

FIGURE 13.—Diagrams of female multiple mosaic, D935K. Black areas, black; stippling, blue with structural defect; shading, faded (?).

It was quite healthy and vigorous, but its flight was lopsided. Autopsy revealed a normal ovary. Only the wings and tail were preserved after description had been completed. Later, an X-ray photograph (figure 14)

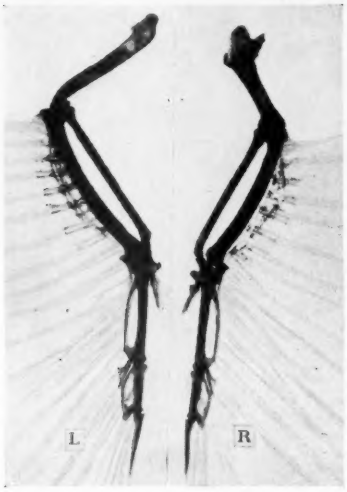

FIGURE 14.—X-ray photograph of left and right wings of multiple mosaic female D935K.

showed that the skeleton of the right wing was nearly 2 cm shorter than that of the left. Comparison of wing bone measurements of left and right wings and with a normal bird gave the following results:

	Normal bird	Mutant female, D935K	
		Left	Right
Humerus	46 mm	45 mm	35 mm
Ulna	53 mm	54 mm	48 mm
Digit (to last joint)	54 mm	49 mm	48 mm
Totals	153 mm	148 mm	131 mm

The feathers also of the right wing were shorter than those of the left; for example, the third left primary measured 183 mm, while the third right measured 170 mm; and the third left rectrix measured 130 mm, while the third right measured 125 mm. Furthermore, the feathers of the right side, and almost entirely in the regions of *s* coloration, were defective in structure, webbing rather poorly.

The color distribution of the bird is shown in figure 13. White spotting occurs only on the left side, which otherwise is spread black, except for the scapulars; these appear to be "faded." On the right side most of the greater feathers show longitudinal blue stripes of defective structure; often there are stripes of black with normal structure (figure 15). The

first two primaries are possibly of the "faded" color, and are also defective in structure.

The degree of asymmetry of white spotting in this specimen is very unusual and probably significant. None of the numerous sibs produced in the mating even approached this degree of asymmetry nor has it been observed in a great number of birds of similar type.

FIGURE 15.—A primary wing feather from the right wing of D935K. Note the even edge of the longitudinal structural fault zone.

The parents were healthy and quite normal; they were breeding regularly and excellently throughout the season when D935K appeared. The father was a common pigeon with a little white spotting and heterozygous for the sex-linked dominant factor *Of*, "faded." The mother was black (heterozygous for *S*), with a little white spotting; she was by pedigree 1/32 *Columba guinea*, but no remaining traces of this species were apparent, and fertility was quite normal. Further investigation of the ancestry has revealed no source for the structural anomalies in this mosaic. It is, however, quite obvious that this case cannot be explained by simple point mutation. Undoubtedly, more extensive chromosomal aberration is involved.

7. *Involving Silky (L)*

SPRUIJT (1931) described a Dutch Cropper pigeon with silky plumage, except for a few normal feathers in the tail and on the legs. Silky plumage is otherwise unknown in this breed. The specimen was mated with a sister, but produced only normal young. The possibility that a somatic mutation to the dominant silky factor, *L*, occurred is strengthened by the fact that a silky individual arising from normal parents presumably by germinal mutation, has been observed (COLE and HOLLANDER 1939).

THE GENETIC BASIS FOR CHIMERISM

No single explanation appears capable of accounting for all the chimeras. In other birds, such as chickens, budgerigars and finches, chimeras of fundamentally differing genetic basis have been reported by CREW and MUNRO (1938). Often simple loss of some kind in heterozygotes is a sufficient explanation. In other cases, non-disjunction has been postulated,

as for example in gynandromorphism or in birds not genetically heterozygous, or where size or other changes are involved. Somatic segregation was suggested by ASMUNDSON (1938) to explain a chimeric turkey. Such an explanation could possibly apply to some of the pigeon chimeras. Dominant mutation or polyspermic effects have not been suggested in other birds; WRIGHT and EATON (1926) found a dominant somatic mutation in a guinea pig, and polyspermy has been found to occur in *Drosophila pseudoobscura* by CREW and LAMY (1939).

TABLE 2

Summary of Chimeras.

		Mutant Area
1. Involving ash-red (B^A)		
LYELL (1887)	?	Simple recessive, B^A to B
2067B	♂	Simple recessive, B^A to B
2411A	♂	Simple recessive, B^A to B
LEVI AND HOLLANDER	♂	Simple recessive, B^A to B
LEVI AND HOLLANDER	♂	Simple recessive, B^A to B
E284E	♂	Simple recessive, B^A to B
2721E	♂	Complex; 2 factors involved
2. Involving chocolate (b)		
LEVI AND HOLLANDER	♂	Simple recessive, B to b
3. Involving dilution (d)		
KEESLING (1924)	♂	Simple recessive, D to d
HOLLANDER	♂	Simple recessive, D to d
4. Involving grizzle (G)		
LEVI AND HOLLANDER	♀	Simple recessive, G to g
5. Involving recessive red (e)		
(a) with recessive mutant areas		
METZELAAR (1926)	♀	Simple recessive, E to e
2708.1	♂	Simple recessive, E to e
2708.2	♂	Simple recessive, E to e
1158H	♀	Simple recessive, E to e
2688H	♂	Simple recessive, E to e
(b) with dominant feathers		
1875C	♂	Dominant, e to E
1057A	♀	Dominant, e to E
T49D	♂	Dominant, e to E
T55C	♂	Dominant, e to E
878A	♂	Dominant, e to E
1470R	♀	Dominant, e to E
925B	♂	Dominant, e to E
6. Involving S ("spread black")		
Chicago "half-sider"	?	Simple recessive, S to s
D935K	♀	Complex
7. Involving silky (L)		
SPRUIJT (1931)	♂	Dominant, l to L

For purposes of ready reference the chimeras described in this paper are summarized in table 2.

Simple recessive mutation, without the necessity of invoking chromoso-

mal loss, somatic segregation or other aberrancy, will explain the majority of the pigeon chimeras; mutation from recessive to dominant will explain all the others except 2721E and D935K, which are more complex. The fact that all the chimeras involving sex-linked characters are males may indicate that the explanation is really not so simple. Moreover, factors are involved which are ordinarily stable (*B* and *D*) as well as one (B^4) which, according to the flecking results, is regularly labile.

Polyspermy might possibly account for 2721E (group 1) and the reds with small black areas (group 5, b) but the results of polyspermy should be detectable in almost any mating of heterozygote with recessive homozygote and hence it cannot be very prevalent. Evidence for it has not been found in hundreds of suitable matings. Furthermore, there appeared to be some tendency for the black-on-red condition to occur in related birds. In this respect it differs from the other chimeras.

D935K (group 6) is the only known chimera in pigeons involving body structural characters and, in this case, feather structure as well. Apparently a single loss can account for this case only if *S* and white spotting, and presumably factors for structural development as well, are assumed to be on the same chromsome. There is no independent evidence to support this assumption. It would appear likely, however, that in this case a whole autosome, or more, was eliminated early in the development of the embryo.

The somatic nature of all the above pigeon chimeras is evidenced by failure of the condition to be transmitted in all cases when breeding tests were made. The tests were not always conclusive, it is true, but none gave any indication that the gonads were affected, even when the major portion of the body appeared "mutated," as in 2411A (see figure 3).

White spotting was present in at least 10 of the 26 chimeras described but probably as an incidental association. There are many essentially symmetrical patterns of white spotting in pigeons which breed more or less true. Although the developmental and hereditary basis for white spotting has not yet been successfully analyzed, it seems safe to conclude that it is not chimerism of the above sort because of its relatively predictable inheritance. Such marked asymmetry of the white as occurred in D935K is altogether unusual and is undoubtedly part of the mosaic pattern in this bird. This is the only specimen of this type that has come to our attention, but possibly the "scherzo" pigeons mentioned by Italian writers, as quoted by HOLMES (1921), are of this sort.

THE DEVELOPMENTAL BASIS OF CHIMERISM

No obvious law has been found governing the distribution of the differing areas in chimeras. In other species of birds CREW and MUNRO (1938)

found that sharp left-right asymmetry was common, but in pigeons we have found only one such case. Blotchy effects are most common and to a certain extent comparable cases may be cited in such forms as guinea pigs and Drosophila. In some cases the mutant areas are of more or less central location (for example, 2688H, group 5, and 2411A, group 1); in METZELAAR'S mosaic (group 5) they are distal. Only the nape of the neck and the interscapular region seem to be affected with any considerable regularity.

The interpretation of these heterogeneous arrangements in terms of development is not easy, especially as cell lineage in the definitive differentiation is obscure. Also, in considering color, the peculiar behavior of the pigment cells must be considered. Studies in this field, as summarized by DANFORTH (1939), indicate that in the embryo the "pigmentoblasts" have neural origin and migrate to their final positions. If the migration is irregular it may help to explain the irregularity of chimerism. At any rate, several discontinuous areas are presumably not to be interpreted as due to separate mutations. It would be difficult to explain why, considering the rarity of chimeras, a bird which had one mutation in its early development should have several simultaneous ones.

Unlike flecking, the color arrangements in chimeras have proved permanent through successive molts except in one case (reported by LEVI and HOLLANDER, group 1). In this case part of the mutated feathers in the tail changed in a reverse direction, back to the dominant color. We have no explanation of this case, but until more is known concerning color determination we may assume that it might harmonize with genetic interpretation.

In the *S* mosaics (group 6) clear striping effects, both of color and structure, were noted. The striping is more clean-cut than in flecking, and fits excellently the theory of axial cell-lineage in the feather. The *S* factor affects not pigment quality, but the arrangement or pattern of the pigment in the cell, and this may well be determined by the tissues in which the pigment cells reside rather than by the pigment cells themselves. The tissue cells having no tendency to wander in the growing feather, mosaicism would be expected to show up as sharply distinguished axial striping, and the same is true of a structural difference.

This study of chimeras in pigeons demonstrates that the factors involved are autonomous in development, that is, two different alleles are able to express themselves clearly in the same bird. Where the differing areas adjoin, there may be mixture, but no true blending of colors or structural differences. Because of this independence chimeras may have certain advantages over the transplantation technique for the study of tissue relationships, since no tissue antagonism is to be expected.

GENERAL DISCUSSION

Comparison of flecking and chimerism

Flecking, like most cases of chimerism in pigeons, consists of irregular areas of recessive color in heterozygous birds which otherwise are of the dominant phenotype. The flecks rarely involve whole feathers but consist of patches on the feather, often of very small size. This would seem to indicate that the mutations occur late in ontogeny, indeed within the individual feather germs. The mutant areas in chimeras, on the other hand, must have their origin in mutations which have occurred much earlier, possibly in some cases even as early as the first cleavage of the egg (figures 10 and 11). If it occurred still earlier, namely in the germinal tissue of the parent, a mutant *individual* might result, and it is possible that Feldman's original "faded" came about in this way (see p. 18).

Mutations at the early stages which produce chimeras are relatively rare, even with genes, such as B^A, which show high inconstancy in the feather follicles. It must be supposed, therefore, that there is some internal condition at this late stage which predisposes to a high frequency of mutation in certain labile genes. Furthermore, the tendency would appear to increase with age, as indicated by the greater amount of flecking in old birds. The mutant areas in chimeras, on the other hand, have not been observed to change with successive molts,[3] which would indicate again that the spot is determined by a single earlier mutation.

The difference between flecking and the larger areas of the chimera is usually clear enough but the actual size of the spot in a chimera, as shown in E284E, figure 4, may be smaller than a large single-feather "fleck" (fig. 1). The difference is that the former covers an area of several feathers, so it is obvious the causative change ("mutation") must have occurred before differentiation of the individual feathers. Furthermore, even when it is small, the chimera spot appears to be constant through successive molts.

Mutation or physiological control

That internal physiological conditions may normally determine whether or not a gene will exhibit its characteristic phenotypic expression is definitely shown in an ordinary Barred Plymouth Rock feather, for example, where the action of the gene is alternately expressed and suppressed. The work of LILLIE and his associates has demonstrated that not only barring, but other patterns as well may be controlled with considerable accuracy by changing physiological conditions. It is not to be supposed in either of these cases that there has been corresponding genetic change in the cells concerned. Probably the same is true for all regular patterns. It has been

[3] One "reverse" exception, group 1, p. 25.

suggested that in the case of piebald patterns the white areas may be regions of somatic genetic change, but there seems to be no direct evidence in favor of such an explanation. Why then may not the flecking and chimerism described in this paper be explained as direct physiological response to local internal environment rather than to localized genetic change? Probably the strongest argument at the present time for the latter interpretation is the great irregularity of occurrence of the flecks and spots and the way they fit into a logical genetic scheme. Furthermore, the initial changes, particularly in the case of the larger chimera spots, must have occurred well back of the point where they are expressed, and they are not synchronized as the responses to direct physiological conditions so commonly are. The "half-sider" described and illustrated (figures 10 and 11) could scarcely be referred to a general physiological differentiation in the first two blastomeres, which was so permanent as to continue through all subsequent somatic cell generations. Such a result would, however, naturally follow from a genetic mutation in one of these blastomeres. From the half-sider to the bird with ordinary flecking there seems to be a series, representing mutations at different stages of development, which make it logical to apply the same interpretation to the latter type of marking. This does not preclude the possibility that changes in internal environment, such as that accompanying ageing, may have an influence on the frequency of somatic genetic change.

SUMMARY

Three sex-linked color factors in the pigeon, all probably allelic, and each dominant to wild type, are found accompanied in heterozygous males and the hemizygous females by a sort of variegation which we have termed flecking. This is usually most marked in males and in aged birds. Large flecks tend to be repeated in successive feathers from the follicles; small flecks less noticeably so. Flecks of recessive color have also been observed in connection with an autosomal factor, grizzle, but no special study of this condition has been made.The assumption of frequent recessive somatic mutation seems to account adequately for flecking.

Chimeras are treated in seven main groups based on the factors involved. The areas in chimeras which are discordant with the regular phenotype of the bird are commonly larger than the flecks, involving considerable patches of feathers. They may even include as much as half the bird, as in a "half-sider" described. Like the flecks, they mostly occur in heterozygotes, and in the majority of cases are recessive. These cases can also be explained as the result of recessive somatic mutations which have occurred relatively early in the ontogenetic development.

A few of the chimeras are not susceptible of this simple explanation.

These appear to involve dominant mutation, or possibly polyspermy. One multiple chimera described involves at least two color effects and two structural effects (counting arrangement of pigment as structural). It is suggested that at least one autosome was lost in this case.

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AN ANALYSIS OF X-RAY INDUCED CHROMOSOMAL ABERRATIONS IN TRADESCANTIA

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THE cause and nature of chromosomal alterations has become an important genetic problem since it has been shown that both mutation and speciation often are associated with structural alterations of the chromosomes (DOBZHANSKY 1937). The frequency of spontaneous structural changes is too low in most organisms to permit a statistical analysis of types or frequencies of alterations, although recently GILES (1940) has been able to analyze a considerable number of natural chromosomal aberrations in Tradescantia hybrids. In most respects these natural aberrations resemble those induced by X-rays, and although the spontaneous breaks are not caused by radiation, the same secondary factors seem to be involved in producing the various types and frequencies of aberrations. The X-ray induced aberrations can be produced in large numbers under controlled conditions, permitting adequate statistical analyses of types of aberrations and their frequency in relation to dosage, temperature, time of exposure, and radiation intensity.

The microspores of diploid species of Tradescantia provide excellent material for an analysis of X-ray induced chromosomal aberrations. The six chromosomes are large and the nuclear cycle can be timed rather accurately. During the summer months the microspore nucleus remains in the resting stage for about five days after the microspore is formed following meiosis. At about 30 hours before metaphase the chromosomes begin splitting, and at 23 to 24 hours before metaphase all chromosomes are split to form the sister chromatids. Thus the nuclear cycle from microspore formation to metaphase requires about six days. During the winter months the nuclear cycle is nearly twice as long and the prophase stage may begin at about 60–70 hours before metaphase. For an analysis of chromatid breaks induced at prophase the cells were fixed 24 hours after raying. Microspores fixed five days after raying were irradiated in the resting stage. Preparations were made at 48 hours after raying during the winter months, and 30 hours after raying during the summer months, for the analysis of very early prophase stages. The analyses of dosage, the time factor, and temperature effects were based on experiments done during the winter months. The division figures obtained provide a true random sample of aberrations because there is no chance of elimination of chromosomes or chromosome fragments.

The microspore cells were smeared, fixed in alcohol-acetic for a few

minutes, and stained with aceto-carmine. Several hundred division figures usually were obtained from each flower bud, and preparations with less than about a hundred metaphase or anaphase figures were discarded. Since there are six chromosomes in each microspore each preparation permitted the anlysis of from 600 to 2000 chromosomes. The results to be presented are based on an analysis of more than 300,000 chromosomes.

The statistical treatment of the data presents a difficult problem. Most of the individual values given in the various tables are based on the average frequencies on three slides, and the total number of chromosomes is from 3,000 to 6,000. Neither the number of slides nor the number of chromosomes provides the correct value of n for a critical analysis of variation. Accordingly we have taken the deviation from the mean, in percent of the mean, for each series of observations involving three or more slides each, and have obtained the standard deviation and probable error from these figures. The value of n was taken as the number of series times $n-1$ slides examined for each series. The generalized probable error is found to be about 10 percent of the mean. Many of the experiments were repeated several times with comparable results.

TYPES OF CHROMOSOMAL ABERRATIONS

Two general types of chromosomal aberrations are induced by X-rays, namely, chromatid breaks, and chromosome breaks. The chromatid breaks are induced at prophase when each chromosome consists of two sister chromatids, while the chromosome breaks are those induced during the resting stage when the chromosomes are in the form of single threads. Each of these general types of aberrations may be further classified into two groups: the aberrations caused by a single break which are referred to as one-hit breaks, and aberrations involving breaks in two different chromosomes or different loci of the same chromosomes which are referred to as two-hit aberrations. The origin and nature of these four classes of chromosomal aberrations is shown diagramatically in figure 1.

The one-hit chromatid breaks may involve one or both chromatids. The terminal deletion of one of the two chromatids (Plate I, figs. E and F) results in a chromatid fragment which is usually not included in either daughter nucleus at telophase. Often, however, the fragment appears to be separated from the centric arm only by an achromatic lesion, suggesting a partial breakage or imperfect reunion. Although these one-hit terminal deletions are rather frequent they cannot be scored accurately and have not been included in the analysis of X-ray breaks. The most frequent type of one-hit chromatid aberration is a break in both chromatids at the same locus followed by lateral fusion to produce a dicentric chromatid and an acentric U-shaped fragment (Plate I, B). At anaphase the dicentric chro-

matid forms a single bridge and the fragment tends to straighten out (Plate I, C). Such fragments are rarely included in the daughter nuclei. The ends of the fragment are the normal ends of sister chromatids (cf. figure 1).

The two-hit chromatid aberrations are of various types. The most frequent types are fusions between broken chromatids of two different chromosomes. These consist of reciprocal chromatid exchanges, producing a

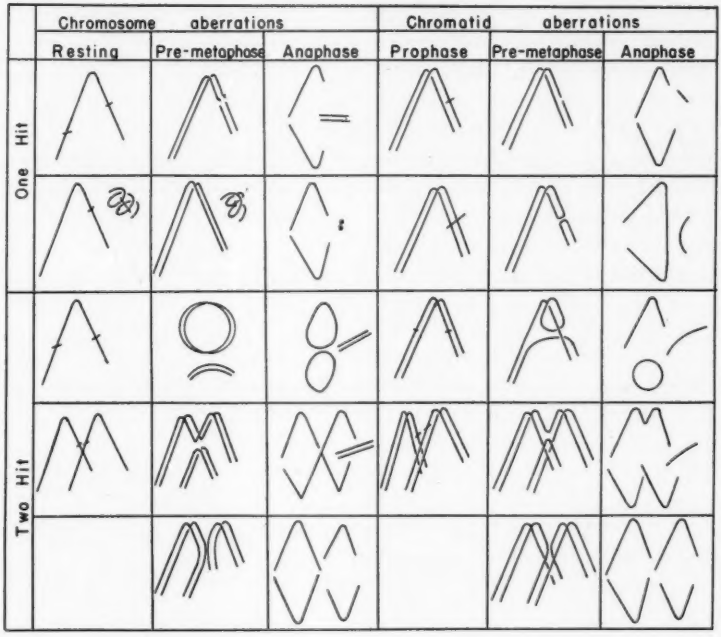

FIGURE 1.—The types of chromosomal aberrations induced by X-rays at prophase and at the resting stage. Only the types which can be recognized at metaphase or anaphase have been included, since they are the types used in the various analyses.

pseudo-crossover type of configuration (Plate I, D), or the broken ends may fuse to form a chromatid bridge between two chromosomes accompanied by an acentric fragment (Plate I, E). Other types have been found but they are rare. Occasionally the corresponding arms of a single chromosome are of unequal length at anaphase (Plate I, F) indicating an intercalary duplication such as KAUFMANN and BATE (1938) have described in Drosophila. Fusions between breaks in each of the two arms of a single chromosome have produced a few chromatid rings, and in rare cases an acentric ring is produced from two breaks in a single arm.

The chromosome aberrations include both one-hit and two-hit types. The one-hit aberrations include both intercalary and terminal deficiencies. The loss of a terminal segment of a chromosome results in a shortened arm with no fusion of the ends of sister chromatids, and the acentric fragment consists of two separate chromatids at metaphase (Plate I, G). The small intercalary deletions presumably are deficiencies produced by breaks in adjacent relic spirals during the resting stage (figure 1). These deleted fragments are small (Plate I, G and N) and have not been included in the analysis of chromosome breaks. Most of these deficiencies have been found to be two-hit aberrations (Rick, unpublished observations).

The two-hit chromosome aberrations include reciprocal interchange or fusion between different chromosomes and fusion or exchange between the arms of the same chromosome. Occasionally a relatively large intercalary deletion is produced resulting in an acentric ring fragment. Reciprocal interchange between arms of different chromosomes can be detected only when the exchange is unequal (Plate I, J), and such exchanges are infrequent. Fusions between broken ends of two chromosomes result in a dicentric chromosome and an acentric fragment (Plate I, H and I). The

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DESCRIPTION OF PLATE I

Photographs of Tradescantia microspore chromosomes showing the types of aberrations induced by X-rays. Compare with diagrams in figure 1.

FIGURE A.—Untreated,—six normal chromosomes at metaphase.

FIGURE B.—A one-hit chromatid aberration induced at prophase. Lateral fusions have occurred between the ends of the broken sister chromatids to produce a shortened arm with terminal fusion of sister chromatids and a U-shaped acentric fragment. Metaphase.

FIGURE C.—The same at anaphase showing the chromatid bridge and partially straightened U-shaped fragment.

FIGURE D.—A two-hit chromatid aberration. Reciprocal interchange between chromatids of different chromosomes.

FIGURE E.—The same, but with chromatids fused to form a chromatid bridge between chromosomes. Also a one-hit chromatid aberration—terminal deletion of only one of the two sister chromatids.

FIGURE F.—An intercalary chromatid duplication and deficiency involving the sister chromatids of one arm. Results in unequal arms at anaphase. Also a single chromatid terminal deletion.

FIGURE G.—Two one-hit chromosome aberrations. One is a terminal deletion of most of one arm, and the other a very small interstitial deletion.

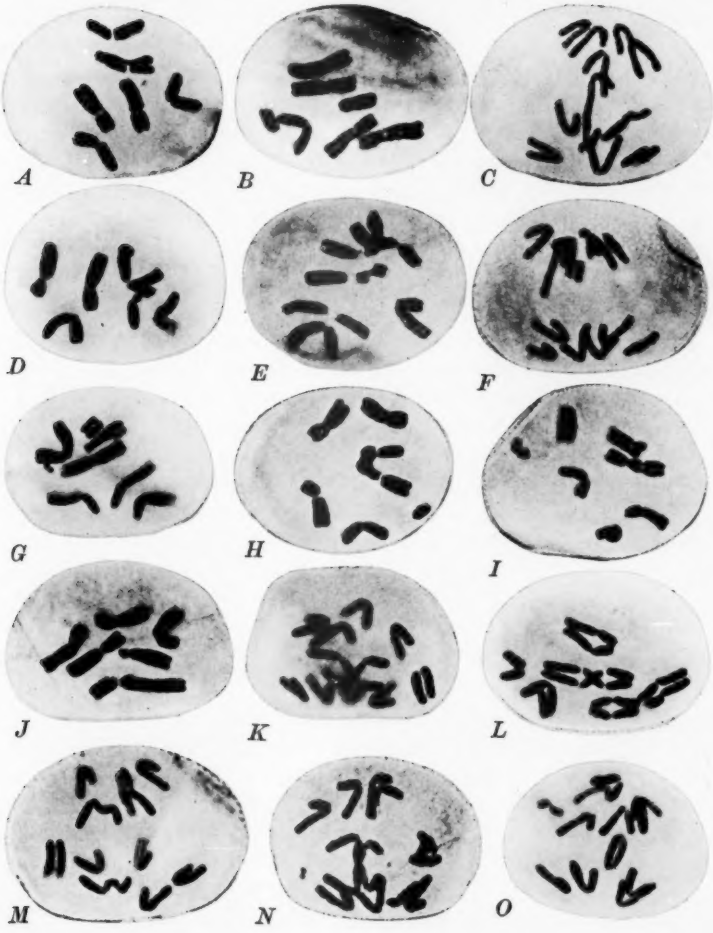
FIGURE H.—A two-hit chromosome aberration. A dicentric chromosome and the accompanying fragment. Note relational coiling between centromeres.

FIGURE I.—Two two-hit chromosome aberrations. A dicentric and a locked ring with their accompanying fragments.

FIGURE J.—Unequal interchange between two chromosomes.

FIGURES K–N.—Behavior of dicentric chromosomes at anaphase. Also a terminal chromosome deletion and an interstitial deletion in figure 14.

FIGURE O.—A continuous ring chromosome at anaphase. Also somatic non-disjunction of two anaphase chromosomes.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broken ends of the terminal deletions fuse to produce a fragment consisting of parts of two chromosomes. The ends of the fragment are the normal ends of the two arms. The relational coiling between centromeres of the dicentric chromosome persists to metaphase and at anaphase the dicentric chromatids may separate freely, form an X-shaped bridge, or interlock (Plate I, K–N). Fusion between breaks in the two arms of the same chromosome results in a ring chromosome and an acentric fragment (Plate I, I). The ends of the fragment are the normal ends of the two arms. These ring chromosomes may separate freely at anaphase, they may be locked, or they may form a single large ring (Plate I, O). Occasionally a ring chromosome is locked around its rod fragment.

Combinations of chromosome and chromatid breaks may occur in the same cell or even in the same chromosome, but they are very rare. The individual chromosomes must split very rapidly at prophase (SAX and MATHER 1939).

Other types of chromatid and chromosome aberrations undoubtedly occur, but inversions, transpositions or intra-chromosomal translocations, and simple intercalary translocations, either cannot be detected, or cannot be distinguished from reciprocal translocations. The types of aberrations included in the subsequent analyses consist of the one-hit chromatid breaks involving both chromatids, two-hit chromatid breaks which produce exchange or dicentric chromatids, and the two-hit chromosome breaks resulting in dicentric and ring chromosomes. These constitute most of the visible chromosomal alterations induced by X-ray doses used in the following experiments.

FREQUENCIES OF TYPES OF ABERRATIONS IN RELATION TO SPATIAL ARRANGEMENT OF THE CHROMOSOMES

The relative frequencies of one-hit and two-hit aberrations vary with the X-ray dosage and will be considered later. The various types of two-hit aberrations, both chromosome and chromatid, appear in the same relative frequencies at different dosages, even though the actual frequencies increase exponentially with dosage. The relative frequencies of the two-hit aberrations provide evidence regarding the conditions and limitations of fusion between broken ends of chromatids and chromosomes. The frequencies of types of chromatid and chromosome alterations are shown in table 1. The dicentric chromatids are more than twice as frequent as the exchange chromatids. The exchange types cannot ordinarily be recognized at anaphase but most of these observations were made at metaphase so that the 2:1 ratio is approximately correct. The ratio of dicentric and ring chromatids is about 13 to 1, but for the chromosome aberrations the ratio of dicentrics and rings is 3.3 to 1.

The loci of breaks

Usually the exchange breaks are at similar loci in respect to the centromeres of the two chromosomes. An analysis of a random sample of dicentric and exchange chromatid aberrations showed 33 with breaks in both chromatids at about the same relative loci and only six which involved breaks at clearly different loci in the two chromosomes. A similar condition must obtain for chromosome breaks because unequal translocations are seldom observed.

The distribution of X-ray induced breaks must be at random among the chromosomes and at random for the loci of any one chromosome. But the

TABLE 1

Frequencies of types of chromatid aberrations; 24 hours, 40–200 r.

TOTAL CHROMOSOMES	ONE HIT		TWO-HIT BREAKS					
	DELETION	%	EXCHANGE	%	DICENTRIC	%	RING	%
9906	468	4.7	182	1.8	402	4.1	30	0.3

Frequencies of types of chromosome aberrations; 5 days, 100–500 r.

TWO-HIT ABERRATIONS			
TOTAL	DICENTRICS	RINGS	RATIO
10,344	7,936	2,408	3.3:1

aberrations resulting from these breaks are not at random for the various loci of the chromosome arms. The frequency of both chromosome and chromatid breaks which result in aberrations is greatest at the proximal end of the chromosome arm. The distribution of such chromatid and chromosome breaks is shown in graphic form in figure 2. The data were derived from an analysis of 340 chromosome aberrations and 334 chromatid aberrations (SAX and MATHER 1939). The loci of breaks were divided into five groups; group one including those in the proximal fifth of the chromosome arm and the subsequent groups including the more distal loci. The difference in distribution of chromatid and chromosome breaks is statistically significant according to the χ^2 test, but technical difficulties in determining breakage points in dicentric chromosomes may account for this difference.

THE RELATION BETWEEN X-RAY DOSAGE AND FREQUENCY OF CHROMOSOMAL ALTERATIONS

The relation between the X-ray dosage and mutation rate in Drosophila has been shown to be linear by a number of investigators (TIMOFÉEFF-

RESSOVSKY 1937). Recent work on both Drosophila and Tradescantia shows that the relation between X-ray dose and chromosomal aberrations is not linear. OLIVER (1932), using genetic methods, was the first to show an exponential increase in chromosomal alterations with increased dosage in Drosophila. Direct cytological analysis of the salivary gland chromosomes of Drosophila also shows an exponential increase in chromosome changes with dosage (BAUER, DEMEREC and KAUFMANN 1938). MULLER (1938) also refers to experiments which indicate that the frequency of both translocations and inversions varies as the 3/2 power of the X-ray dose. The frequency of chromosome aberrations in Tradescantia plotted against dosage also shows an exponential curve (SAX 1938).

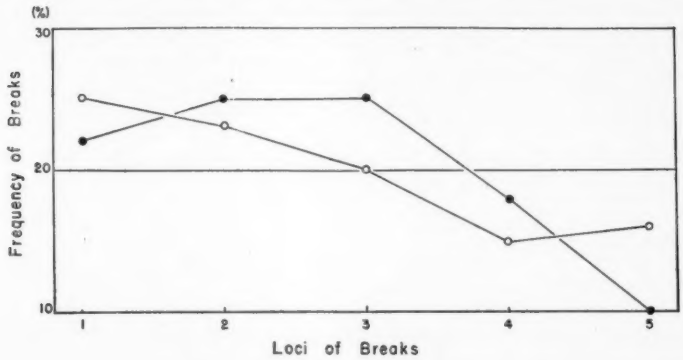

FIGURE 2.—Loci of chromosome and chromatid breaks involved in X-ray induced aberrations. Locus 1 includes the first fifth of the chromosome arm next to the centromere and locus 5 the distal fifth. If such breaks were at random 20 percent of all breaks should be found at each of the 5 loci. Data from SAX and MATHER (1939). ○ = chromatid. ● = chromosome.

In all these experiments the frequency of chromosome aberrations increased approximately as the 3/2 power of the dosage. If each aberration is dependent upon two breaks, as is probably true in all these experiments, the frequency of aberrations should increase as the square of the dosage. In all these previous experiments the dosage was increased by increasing the time of exposure. Recently it has been found that the frequency of two-hit chromosomal aberrations is dependent upon the time-intensity factor, and that a high intensity of dosage is more effective in producing aberrations than the same dose given slowly (SAX 1939). Accordingly we should vary the dosage by varying the intensity rather than by varying the time of exposure.

In the following experiments on the relation between X-ray dosage and frequency of chromosomal aberrations, the dosage was varied by changing

the distance between the target and the flower buds according to the inverse square law, so that variation in dosage was given without changing the exposure time. The minimum distance used was 24 cm, but even at this distance there is some variation in dosage, due to slight differences in the positions of the young buds in the inflorescences. At greater distances this experimental error is decreased. The source of the X-rays was a Coolidge tube with a tungsten target, operated at 10 ma, 134 K.V. with no screen.

The results of increased dosage on frequency of chromosomal aberrations induced at the resting stage are shown in table 2. The frequency of these

TABLE 2

The relation between X-ray dosage and chromosome aberrations; time constant.

DOSE IN R UNITS	TOTAL CHROMOSOMES	CHROMOSOME ABERRATIONS*				
		DICENTRIC	RING	TOTAL	%B	$(D/107)^2$
100	1800	5	1	6	0.7	0.8
200	1710	21	8	29	3.4	3.5
300	1464	48	13	61	8.3	7.8
400	1902	104	28	132	13.9	14.0
500	1530	134	25	159	20.8	21.8

* Each aberration involves two breaks.

two-hit aberrations increases as the square of the dosage. The equation for the curve is percent breaks, B, equals $(D/107)^{2.0}$, where D is the dosage in r units and 107 is a constant. The values of the exponent and constant were derived by plotting the logarithm of the dosage against the logarithm of the percent of effective breaks and obtaining the values from the graphs.

The low frequency of one-hit chromosome breaks induced at the resting stage does not permit an adequate comparison of the effects of varying dosage on one-hit and two-hit chromosome aberrations, so for this comparison we have used one-hit and two-hit chromatid aberrations induced at prophase. The frequency of chromatid breaks is much higher than that of chromosome breaks and it was not possible to analyze accurately the chromatid breaks induced at a dosage of much over 200 r. On the other hand it was possible to obtain an appreciable number of chromatid aberrations at 10 r. The effect of varying dosage on both one-hit and two-hit chromatid aberrations is shown in table 3. The frequency of the two-hit chromatid aberrations increases approximately as the square of the dosage. The equation for the curve is percent $B = (D/67)^{1.9}$. The one-hit aberrations, however, show an approximately linear relation to dosage, the equation being percent $B = (D/45)^{1.1}$. The deviations from the expected value

of the exponents may be due to experimental error since the data in this table were derived from three different series of experiments extending over a period of several months. It is possible that environmental conditions or the output of the X-ray tube may have varied sufficiently so that

TABLE 3

The relation between X-ray dosage and one-hit and two-hit chromatid breaks; time constant.

DOSE IN R UNITS	TOTAL CHROMOSOMES	CHROMATID ABERRATION BREAKS					
		1-HIT	%B	$(D/45)^{1.1}$	2-HIT	%B	$(D/67)^{1.9}$
10	6330	14	0.2	0.2	2	0.03	0.02
20	6930	24	0.4	0.5	4	0.06	0.08
40	8610	76	0.9	1.1	34	0.4	0.31
80	7368	148	2.0	1.9	112	1.5	1.4
120	4902	141	2.9	2.9	124	2.5	3.0
160	8292	375	4.4	4.0	422	5.1	5.3
200	8508	472	5.1	5.1	685	8.1	8.1

the data from different experiments were not strictly comparable. It is also possible that some of the one-hit types of aberrations were produced by two adjacent independent hits. In general, however, it is apparent that "one-hit" aberrations show a linear response to dosage, while the "two-hit" aberrations vary as the square of the dosage, when the exposure time is held constant.

The effect of increased dosage on the frequencies of the various types of aberrations is shown in the form of curves in figure 3. The curves for the chromatid aberrations have been extrapolated beyond 200 r for comparison with the two-hit chromosome aberration curve. The frequency of the two-hit chromatid breaks is considerably greater than that of the two-hit chromosome breaks. The two frequencies are not entirely comparable, however, because the reciprocal interchanges can be detected when induced at prophase, but only rarely when induced at the resting stage.

The relative frequencies of one-hit and two-hit chromosomal aberrations vary not only with dosage, but also with the time of prophase development at the time of irradiation. At very early prophase the exchange or fusions between chromatids of different chromosomes are much less frequent in relation to the number of terminal deletions than at later prophase stages. The relative frequencies of these one-hit and two-hit chromatid aberrations and the two-hit chromosome aberrations induced at early prophase, 48 hours before metaphase, are shown in table 4. The theoretical frequency of two-hit chromatid breaks is based on the relative frequencies of one-hit and two-hit aberrations produced at mid-prophase, while the theoretical frequency of the chromosomal aberrations is derived from the equation

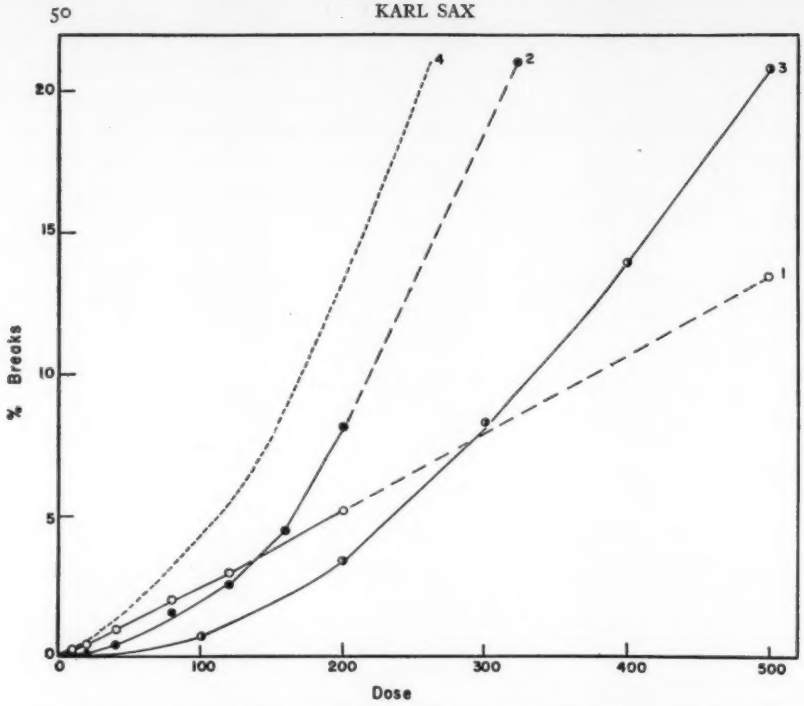

FIGURE 3.—The relation between the frequencies of chromosome and chromatid aberrations and the X-ray dosage in r units. Time of exposure constant. The curves for the one-hit and two-hit chromatid breaks have been extrapolated beyond 200 r. The equations for the curves are: (1) one-hit chromatid breaks, percent $B=(D/45)^{1.1}$, (2) two-hit chromatid breaks, percent $B=(D/67)^{1.9}$, (3) two-hit chromosome breaks, percent $B=(D/107)^{2.0}$ and (4) total chromatid breaks, percent $B=(D/37)^{1.5}$.

percent $B=(D/107)^2$, assuming that all chromosomes are single. The data show that most of the chromosomes were not yet split at this time. The aberrations induced in the split chromosomes show a much lower frequency of two-hit aberrations than would be expected at mid-prophase.

TABLE 4

Frequencies of one- and two-hit chromatid breaks induced at very early prophase; 48 hours.

DOSE	TOTAL CHROMOSOMES	CHROMATID					CHROMOSOME		
		ONE-HIT	%	TWO-HIT	%	THEOR.	TWO-HIT	%	THEOR.
160 r	3276	26	0.8	8	0.2	1.0	90	2.7	2.3
320 r	3030	46	1.5	6	0.2	3.8	252	8.3	9.0

The time-intensity factor

An analysis of the time-intensity factor in the X-ray production of various types of chromosomal aberrations has shown that the two hits necessary to produce an alteration involving two chromosomes need not occur simultaneously, but may occur over a period of time as long as an hour (SAX 1939). The time-intensity factor was varied by giving equal doses at different intensities, or by giving the same intensity of radiation over various periods of time by intermittent exposures. The intensity of radiation was varied by placing the inflorescences at various distances from the target according to the inverse square law. The results of the time factor at different X-ray intensities are shown in graphic form (figure 4). The

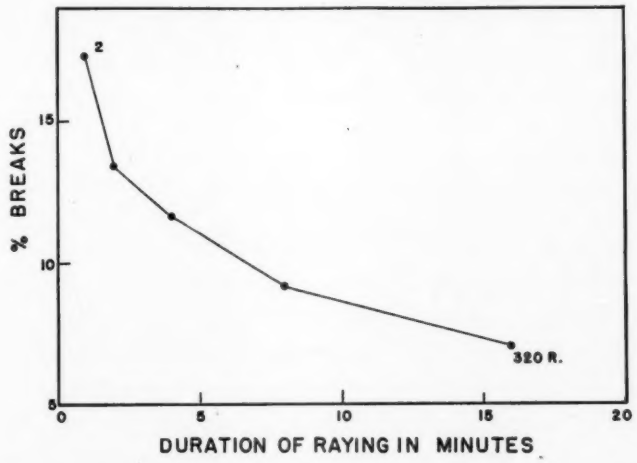

FIGURE 4.—The relation between the frequency of chromosomal aberrations and the intensity of X-radiation. The intensity of radiation was varied from 320 r/m to 20 r/m.

dosage was 320 r and the intensity varied from 320 r/m to 20 r/m according to the time of exposure. The curve is based on analysis of 10,620 chromosomes. The chromosomes which were given 320 r in one minute show twice as many aberrations as those which were given 320 r in sixteen minutes. Similar experiments using other total dosages showed similar results.

Since the chromosome aberrations used in this analysis include only two-hit alterations, the greater frequency of such aberrations at high X-ray intensity must mean that reunion of broken ends occurs within certain limits of time. If the broken ends fuse quickly a low intensity should produce fewer aberrations than a high intensity of radiation be-

cause a break in one chromosome would heal before a second break occurred in an adjacent chromosome, and no aberration would be produced. But if the broken ends remain in an unstable condition for a long time and

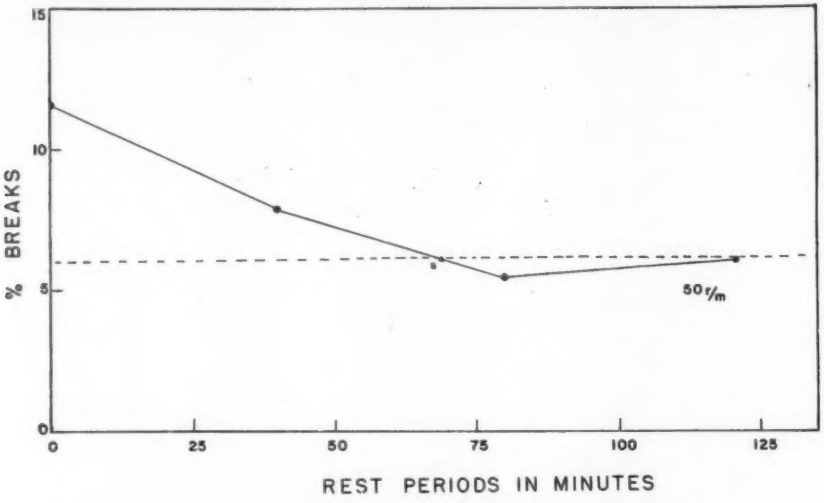

FIGURE 5.—The relation between frequency of chromosomal aberrations and intermittent radiation. Four two-minute X-ray doses were given at 50 r/m, with rest periods of 0, 40, 80 and 120 minutes respectively. The base line (B), established by determining the percentage of breaks for a single two-minute exposure and multiplying by 4, coincides with percent of breaks at about the 70 minute rest period.

few fusions occur during the time of raying there should be little effect of the time-intensity factor. The results show that many fusions occur in a few minutes and probably in even less time, but they do not indicate the maximum length of time necessary to complete the fusion of all broken ends.

The time during which a broken end of a chromosome remains in an unstable condition can be determined from intermittent timing experiments. The results of one of these experiments are shown in the form of a curve in figure 5. Five groups of flower buds were irradiated, all at 50 r/m. The first group was given continuous radiation for eight minutes, the second, third, and fourth groups were given four intermittent exposures of two minutes each, with rest periods of 40, 80, and 120 minutes respectively. The fifth group was given only one two-minute exposure. If broken ends of chromosomes can remain unstable and capable of fusion with other broken ends for only a short time, a sufficiently long rest period should prevent fusions between breaks induced at successive exposures. This point

is reached where a further increase in the rest period produces no further decline in frequency of aberrations. The base line can also be determined by multiplying the frequency of aberrations induced by a single exposure by the number of exposures used in the intermittent timing experiment. In the above experiment the base line is at about six percent and is reached at a rest period of somewhat more than an hour. These results show that a break induced at one exposure period may remain open so that a broken end can fuse with another broken end produced by the second exposure an hour later, although most of the fusions occur in a considerably shorter period. Similar experiments with different X-ray intensities show similar results, although at high intensities, where the dose is relatively high for each exposure, there is less effect of the intermittent exposures. These results are based on an analysis of more than 80,000 chromosomes.

The effect of the time-intensity factor is based on the fact that the two-hit aberrations are dependent upon two independent breaks, limited in time of occurrence. According to this interpretation the time-intensity factor should have no effect on one-hit aberrations. This assumption has been tested by comparing the effect of intermittent timing on one-hit and two-hit chromatid aberrations. As expected, the frequency of the one-hit aberrations is independent of the time factor while the frequency of the two-hit aberrations declines with increasing rest periods (SAX 1939).

THE EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMOSOMAL ABERRATIONS

In an earlier experiment no differences in frequency of chromosomal aberrations were found when the cells were irradiated at different temperatures (SAX 1938). The resu ts were not based on an analysis of individual chromosomes and the frequency of aberrations included all types of alterations obtained at various times after irradiation. More critical experiments show clearly that the temperature during and following irradiation has a marked effect of the frequency of both one-hit and two-hit aberrations (SAX and ENZMANN 1939). The results of two of these experiments are shown in graphic form in figure 6.

The inflorescences were immersed in water at the desired temperature for five to ten minutes before raying and kept just under the surface of the water during irradiation. In a few experiments the flower stalks were removed from the water and placed at room temperature soon after raying, but usually they were left in the water for an hour after raying. When pasteboard cartons were used for containers the temperature dropped gradually during this period, but similar temperature effects were obtained when the flowers were put in thermos bottles where the temperatures were maintained at a constant level during the entire period.

The cells which were held at a low temperature during and following irradiation had from two to five times as many chromosome aberrations as those which were subjected to a high temperature. Preliminary work on the frequency of aberrations at various temperatures shows that the frequency does not decrease regularly with increased temperature, but tends to drop sharply at a critical temperature, which varies from less than 20°C to above 30°C, depending on the time of year the work is done. Presumably this seasonal effect is related to the length of the nuclear cycle; in the winter months the nuclear cycle from meiosis to division of

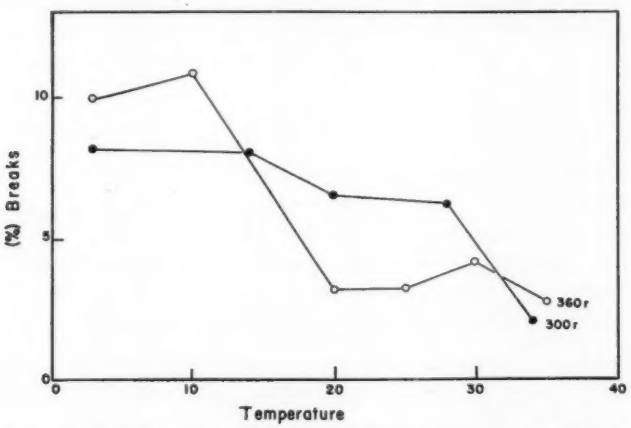

FIGURE 6.—The effect of varying temperatures during and immediately after X-radiation on the frequency of chromosomal aberrations. The different temperatures at which the greatest decrease in frequency of aberrations occur in the two curves is attributed to seasonal effects (Data from SAX and ENZMANN 1939).

the microspore nucleus requires about 12 days and the critical temperature is about 30°, while in late spring the nuclear cycle requires about one week and at this season the critical temperature is less than 20°.

At both mid-prophase and at the resting stage the frequency of X-ray induced aberrations is decreased at high temperatures, but at earliest prophase when the chromosomes are beginning to split into chromatids the higher temperatures cause many more aberrations than are produced by raying at low temperatures. The effect of high (30°C) and low (3°C) temperatures on frequency of X-ray induced aberrations at different prophase stages is shown in table 5. The low frequency of chromatid aberrations induced at early prophase, 48 hours before metaphase, is due to the fact that many of the chromosomes have not split and these produce chromosome aberrations which are not included in this table. The frequency of X-ray induced aberrations, at 24 hours before metaphase, is much higher

in the cold series, but at 48 hours the high temperature produces many more aberrations than the low temperature. This relation holds for both one-hit and two-hit aberrations, although the latter are infrequent in the 48 hour preparations.

INTERPRETATION OF RESULTS

The time of chromosome splitting

The evidence regarding the time of chromosome splitting in the nuclear cycle has been reviewed by MATHER (1937). The results obtained from X-ray experiments are considered to be more critical than the interpretations based on direct visual evidence. Certainly the types of aberrations induced by X-rays at the various stages in the nuclear cycle of Tradescantia microspore indicate that the chromosome reacts as a single thread

TABLE 5

The effect of temperature on frequency of chromatid aberrations at very early prophase (48 hours) and at midprophase (24 hours). Total chromosomes 19,848.

		CHROMATID ABERRATIONS			
		% ONE-HIT	% TWO-HIT	TOTAL	DOSAGE
24 hours	Cold	3.6	2.6	6.2	160 r
	Hot	1.8	1.6	3.4	
48 hours	Cold	0.8	0.1	0.9	
	Hot	1.1	0.4	1.5	
48 hours	Cold	1.5	0.2	1.7	320 r
	Hot	4.6	1.0	5.6	

during the resting stage and becomes effectively split into sister chromatids at prophase (cf. RILEY 1936). It may be argued that the effect of a single X-ray quantum is not sufficiently localized to be used as a tool for differentiating the number of chromatids or half-chromatids in a chromosome, since a single "hit" can break two adjacent chromatids at late prophase when the sister chromatids are more than a tenth of a micron apart. If the chromosomes were double at the early resting stage a single hit would usually break both of the closely associated chromatids, but such breaks should be followed by lateral fusion of broken ends of adjacent sister chromatids. Aberrations of this type are very seldom found in microspores irradiated in the resting stage, and the rare exceptional cases can be attributed to spontaneous aberrations. The effective split which produces differentiated chromatids, corresponding to those involved in crossing over at meiosis, certainly occurs at the prophase stage in the Tradescantia microspores.

There is some experimental evidence that the effective split may occur

at the resting stage preceding nuclear division (MATHER 1937), or even at the preceding anaphase. The behavior of the broken ends of inversion bridge chromosomes suggests that the meiotic anaphase chromosomes may be effectively split. In both Tradescantia (SAX 1937) and Zea (MCCLINTOCK 1938b) the break in an inversion bridge is followed by lateral fusion of sister chromatids to produce a bridge at the following division of the chromosome in the microspore. If the chromosome were single at meiosis a single broken end would have no other broken end with which to fuse, and perhaps such a broken end would remain in an unstable condition until the chromosome is split at the prophase stage of the microspore nucleus, at which time the lateral fusion would occur between the broken ends of sister chromatids. But MCCLINTOCK has found that adjacent double inversion bridges behave in the same way, even though two broken ends are present in the newly formed microspore nucleus. On the other hand a break in a somatic ring chromosome of Zea is followed by fusion of broken ends of the two arms and not between ends of adjacent sister chromatids (MCCLINTOCK 1938a). This difference cannot be attributed to differences in behavior at meiosis and mitosis because X-ray induced aberrations in Tradescantia root tips produce double bridges, which, when broken, are followed by lateral fusion of ends of sister chromatids. There is no reason to believe that the X-ray induced and spontaneous breaks differ in their effects on subsequent fusions of broken ends of chromosomes.

The behavior of the induced ring chromosomes is of interest in relation to the nature of splitting. If these ring chromosomes induced at the resting stage are single threads they must split in different planes because the rings may be continuous or interlocked at the following metaphase. This problem has been analyzed in some detail by HUSTED (1936).

The time of effective chromosome splitting in different microspores is quite variable, ranging from about 23 to 31 hours before metaphase in Tradescantia plants grown in the field during the summer months. The splitting of the chromosomes of a single nucleus is much more limited in time, and the splitting of an individual chromosome must be very rapid since both chromosome and chromatid breaks are rarely found in the same chromosome. There is some indication that effective splitting begins at the proximal end of the chromosome arm and progresses distally (SAX and MATHER 1939).

The frequency of types of aberrations in relation to chromosome arrangement

It has been shown that all potential fusions between broken ends of chromosomes are completed within an hour after the breaks are produced. There can be no extensive movement of the chromosomes during this

time, at most periods in the nuclear cycle, so that fusions between broken ends must be limited to those in close proximity. Certainly at the resting stage there could be no extensive movement of the chromosomes, which in the form of relic coils appear to completely fill the nucleus (BELAR 1928). With an increase in nuclear size and uncoiling of relic spirals at prophase more movement of the chromosomes could occur, but not sufficient to permit random association of broken ends of chromatids. Accordingly, an analysis of the loci of breaks involved in fusions between different chromosomes or chromosome arms should indicate the arrangement and relations of the chromosomes at various phases in the nuclear cycle.

The chromosomes of Tradescantia are strongly polarized during the resting and early prophase stages so that if little chromosome movement occurs between breakage and fusion, the fusions between different chromosome arms should occur usually at corresponding loci in respect to the distance from the centromeres. Such a relation is found for both chromosome and chromatid aberrations. Unequal exchanges between chromosome arms are rare, and an analysis of the fusions and exchanges between chromatids of different chromosomes shows that the breaks have occurred at approximately the same relative loci of both chromosomes in over 80 percent of the figures examined.

The relative frequencies of dicentric and ring chromosomes also show that proximity facilitates, or conditions, the fusions of broken ends of chromosomes. A broken end of a chromosome arm can fuse with a broken end in the other arm of the same chromosome to form a ring, or it can fuse with a break in any one of the ten arms of the other five chromosomes to form a dicentric union. Random reunion of broken ends should produce a ratio of dicentric to ring chromosomes of 10:1, but the observed ratio is 3.3 to 1 (table 1). It is evident that the distance between breaks, both laterally and longitudinally in respect to the polarized arms of the chromosomes, conditions or determines the occurrence of two-hit aberrations.

The spatial relationships of chromosome arms appear to change as the nuclear cycle progresses from the resting stage into prophase. At the resting stage the ratio of dicentric to rings is 3.3:1, but irradiation at prophase results in a ratio of dicentric to rings of 13:1. This decrease in frequency of ring chromosomes at mid-prophase is attributed to the greater separation of the two arms of a single chromosome at this stage. It is known that chromosomes repel each other, especially when they become split into sister chromatids. The prophase chromosomes also become shorter and the increased size of the nucleus permits greater movement of the chromosomes.

The relative frequencies of one-hit and two-hit aberrations induced at prophase vary with dosage as is shown in figure 3. There is, however, a

striking difference in these frequencies between early and mid-prophase (table 4). The relative frequency of two-hit aberrations induced at early prophase is very much less than at mid-prophase. This difference can hardly be attributed to greater repulsion between arms of chromosomes, and must be related to differences in the nature of the induced breaks. The two-hit breaks—dicentrics, exchanges and rings—induced at mid-prophase may depend upon breaks in only one chromatid of each of the chromosome arms involved in the aberrations. The sister chromatids at this stage are distinctly separated and breaks in only one of the two sister chromatids at a given locus are found at metaphase. At earliest prophase, however, the two sister chromatids must be closely associated so that a single X-ray hit would usually break both chromatids at the same locus. Such breaks would be followed by the fusion of adjacent ends of the broken chromatids in most cases, to produce a terminal deletion which is classed as a one-hit aberration. This interpretation is supported by the fact that two-hit chromosome aberrations, induced before chromosome splitting, but at about the same time, show a normal frequency.

The limitations of fusions imposed by the factor of proximity must mean that most of the breaks induced by X-rays do not lead to the production of aberrations. The two broken ends simply reunite in the original position and no structural alteration of the chromosome is visible at later stages in nuclear development. The chromosome breaks which lead to fusions between chromosome arms must constitute only a very small proportion of the total breaks induced, but simple breaks at the following metaphase are far less frequent than aberrations which involve two breaks in close proximity. The one-hit breaks at prophase are frequent only because two chromatids are broken and lateral fusion of broken ends leads to a permanent aberration. The frequency of breaks which heal in the original position cannot be determined, but such breaks must be far more frequent than those which lead to gross structural changes in the chromosomes.

A comparison of the frequencies of types of aberrations has also been used in the analysis of chromosome arrangements in Drosophila sperm (CATCHESIDE 1938; BAUER, DEMEREC and KAUFMANN 1938). The salivary gland chromosomes permit an analysis of alterations within chromosome arms, as well as between chromosome arms, although the numerous cell divisions between irradiation and chromosome analysis must produce differential elimination of the various types of aberrations. CATCHESIDE finds that exchanges within the same arm are twice as favored as those between different chromosome arms. Exchanges between arms of the same chromosome when compared with the frequency of exchanges between arms of different chromosomes, show no significant deviation from a random dis-

tribution. Similar results have been obtained by BAUER, DEMEREC, and KAUFMANN. These results must mean that a single chromosome in the Drosophila sperm is folded so that various parts of the same chromosome arm are in close proximity. Presumably, little movement is possible in the closely packed chromosomes of the sperm and induced aberrations must depend upon close proximity of the induced breaks.

The loci of breaks in relation to aberrations

It has been shown that the distribution of breaks involved in chromosome and chromatid aberrations is not at random for the various loci of chromosome arms. The initial breaks must be at random, but those which are involved in chromosome alterations are most frequent at the proximal ends of the chromosome arms. This distribution must be caused by secondary factors, and since the unequal distribution holds for one-hit chromatid breaks the secondary factor must be confined to the individual arms of the chromosomes. The greater frequency of such breaks at the proximal end of the chromosome arm is attributed to stresses imposed by the various coiling mechanisms which would tend to throw the broken ends out of alignment so that reunion in the original position would be inhibited. The breaks induced in the chromosomes of the sperm of Drosophila show a nearly random distribution of breaks with a possible higher frequency at the distal ends (BAUER, DEMEREC and KAUFMANN 1938). Perhaps the more random distribution of effective breaks in Drosophila can be attributed to the close association of the chromosomes in the Drosophila sperm so that proximity of broken ends does not necessitate any appreciable movement of broken ends in order to produce fusions between different chromosomes.

The relation between X-ray dosage and frequency of chromosomal aberrations

In both Drosophila and Tradescantia the frequency of X-ray induced breaks increases approximately as the 1.5 power of the dosage when the various doses are given at the same intensity. But since the time factor must be considered in such experiments the dosage should be varied by changing the intensity and not the time of exposure. When this is done the frequency of two-hit chromosome and chromatid aberrations in Tradescantia varies approximately as the square of the dosage. This relation should be expected since the chance that two or more independent events will happen together is the product of their respective chances of happening.

The frequency of simple deletions is assumed to be induced by single hits and should show a linear relation to dosage, at least for doses which

produce a low frequency of breaks. The data indicate a slight exponential increase of aberrations with increased dosage which may be due to experimental error, or to the occasional production of the deletions by two independent hits in the two chromatids at approximately the same locus.

The number of X-ray hits or quanta necessary to produce an effect has been calculated by comparing the survival curves with those obtained from Poisson's exponential series (TIMOFÉEFF-RESSOVSKY 1937). For one-hit and two-hit chromosomal aberrations the equation can be expressed as: percent $B = 1 - e^{-x}$ and percent $B = 1 - e^{-x}(1+x)$, respectively, where x represents the number of effective hits. Using an arbitrary value of x of .00025 per r unit of dosage, we find that the calculated values for the one-hit aberrations are practically identical with the observed values. The calculated values of the two-hit curve also are in close agreement with the observed percentages of breaks at the various doses, but in order to get these theoretical values, the value of x must be increased to .0025 per r unit. The dosage curves, $(D/45)^{1.1}$ and $(D/67)^{1.9}$, do not indicate such different dosages for one- and two-hit aberration frequencies, as is indicated by the Poisson exponential series. It is not clear how these apparent discrepancies can be reconciled. It is evident, however, that the slopes of the two dosage curves are practically identical with the theoretical curves for one-hit and two-hit effects.

In all of the various experiments on the relation between dosage and the mutation rate in Drosophila a linear relation has been found. The frequency of mutation is also independent of the time-intensity factor (TIMOFÉEFF-RESSOVSKY 1937). These facts indicate that most of the induced mutations are produced by single hits and that relatively few of the induced mutations are due to "position effects" resulting from two-hit aberrations such as reciprocal translocations and large inversions.

The frequency of X-ray induced aberrations induced in the Drosophila sperm must also depend on the time factor. With dosage controlled by increasing the time of exposure the relation between frequency of aberrations and dosage is expressed by the equation percent $B = (D/K)^{1.5}$ in both Drosophila and Tradescantia; but in Tradescantia, with the time of exposure constant, the frequency of aberrations varies as the square of the dosage. Control of the time factor should also increase the exponent from 1.5 to 2.0 for the dosage-aberration curve in Drosophila.

The differential susceptibility of chromosomes to X-rays during the nuclear cycle and in different types of cells

The frequency of gross chromosomal aberrations is much greater at prophase than during the resting stage, when the same X-ray dose is used (figure 3). The equation for the relation between dosage and frequency of

aberrations is: percent $B = (D/37)^{1.5}$ for all the chromatid aberrations recorded, and percent $B = (D/107)^2$ for the chromosome aberrations. This comparison may not be entirely valid because certain types of aberrations can be detected when induced at prophase, but not when induced at the resting stage. In general, however, the frequency of gross alterations is greatest when the cells are rayed at prophase. This relation is to be expected for the following reasons: there are twice as many threads to be hit at prophase; terminal deletions can be produced at prophase by lateral fusion between broken chromatids while in the resting stage they are produced only when the broken end loses its capacity for fusion; and the secondary factors of chromosome torsion and movement would be expected to be greater at prophase.

A comparison of the frequency of chromosomal aberrations in Tradescantia and Drosophila shows that breaks involving chromosome changes are much more frequent in Tradescantia microspores than in Drosophila sperm. Some of this difference may be attributed to inviable aberrations in Drosophila, since the chromosomes were analyzed many cell generations after raying the sperm. The data of BAUER, DEMEREC and KAUFMANN show that the frequency of breaks, in percent of total chromosomes, may be expressed by the equation, percent breaks $= (D/1150)^{1.5}$. For Tradescantia microspores rayed at the resting stage, with dosage varied by varying the time of exposure, the equation is: percent $B = (D/80)^{1.5}$ (SAX 1938). These data would seem to indicate that the Tradescantia chromosomes are much more sensitive than those of Drosophila. This difference probably can be attributed to differences in chromosome size in the two genera and to differences in chromosome organization in the two types of cells. According to METZ and BOCHE (1939) both chromosome aberrations and mutations in Sciara are less frequent than in Drosophila when given the same X-ray dosage.

The difference in the frequencies of X-ray induced chromosomal aberrations in different cells of the same organism should provide additional evidence regarding X-ray effects on the chromosome. It is known that both mutations and gross chromosomal alterations occur more frequently in the sperm of Drosophila than in the oocytes for a given X-ray dose (OLIVER 1934). In Sciara gross chromosomal alterations are readily secured by raying the sperm, but were not found when the egg cells were irradiated. METZ and BOCHE (1939) attribute this difference in Sciara to physical differences in the chromosomes of the two types of cells. The close proximity of the chromosomes in the sperm should facilitate illegitimate union of broken ends, while most of the chromosomes of the eggs at the time of treatment were in metaphase or anaphase of the first meiotic division. The diffuse stage at meiotic prophase of meiosis also was found to

be resistant to X-ray effects. METZ (1934) has suggested that the chromosome matrix plays an important part in this differential susceptibility of chromosomes to radiation.

The differential susceptibility to X-rays of different stages of the nuclear cycle, of different cells in the same organism, and of similar cells in different organisms may depend in part on the same secondary factors such as proximity of chromosomes, freedom of chromosome movement, and the physical condition of the chromosomes. There is, however, some evidence that differences in the internal structure of the chromosomes may be responsible for differential X-ray susceptibility. NAVASHIN and his collaborators have found that the aging of seeds increases both the mutation rate and frequency of spontaneous chromosomal aberrations (cf. GILES 1940). OFFERMANN (1938) has shown that X-rays produce more mutations in old than in young sperm of Drosophila. These results suggest that aging of chromosomes induces internal changes, probably of a chemical nature, which makes them more susceptible to both spontaneous and induced alterations.

In any analysis of differential susceptibility of different stages in the nuclear cycle it is essential to differentiate the physiological effect of X-rays from the production of mutations and gross chromosomal aberrations which lead to genetic alterations. The physiological effect of suppressed nuclear activity and clumping of the chromosomes has long been known and this reaction in plant cells has been dealt with in considerable detail by MARQUARDT (1938). At moderate X-ray doses the inhibition of nuclear development is temporary and the cell recovers. The time of recovery depends on the dosage (CARLSON 1938, and unpublished). Heavy X-ray dosage may cause so much clumping of the metaphase chromosomes that normal division is prevented and the effect is lethal. Maximum X-ray effects at metaphase probably can be attributed to physiological factors.

It is probable that the physiological and genetic effects can be differentiated by determining the temperature coefficient of the reaction to X-rays. If chromosome aberrations are responsible for cell injury the temperature coefficient should be one or less than one, while if physiological effects are responsible the temperature coefficient should be appreciably greater than one.

The behavior of broken ends of chromosomes

The experimental evidence shows that the induced chromosomal aberrations involving two chromosomes are dependent upon two independent breaks limited in both time and space. The broken ends of chromosomes either reunite in the original position or produce chromosome aberrations within a short time after the breaks are induced by X-rays, except a very small proportion of breaks producing broken ends which appear to have

lost their capacity for fusion. Most of the unions occur within a few minutes, and at the end of about an hour all fusions are completed. This does not necessarily mean, however, that a broken end can remain in an unstable condition for only an hour.

STADLER (1932) has assumed that an acentric fragment may be included in a daughter nucleus by chance and fuse with the original broken chromosome after several cell generations. Such behavior is highly improbable for the following reasons: all potential fusions between broken ends are completed in about an hour in the Tradescantia microspore which has a nuclear cycle of about a week; nearly all the acentric fragments found at metaphase have no broken ends since the two ends of such fragments are the normal ends of chromosomes or chromatids; the few terminal deletions which persist until metaphase appear to have lost their capacity for fusion since chromosome fragments show no lateral fusion of sister chromatids; and very few of the various types of fragments are included in the mitotic daughter nuclei in plant cells. Acentric fragments of meiotic chromosomes often are included in the daughter nuclei in Zea (MCCLINTOCK 1938b), and in the neuroblasts of the grasshopper such fragments usually are included in the daughter nuclei (CARLSON 1938). Even in these cases reunion of acentric fragments is improbable. Moreover, according to MCCLINTOCK, the acentric fragments occasionally included in the Zea microspores act as normal chromosomes both in their cytological behavior and in their genetic influence. Thus there could be no genetic deficiency by deletion of a chromosome segment, or genetic recovery when the fragment joins the centric segment, as STADLER has postulated.

The temperature effect on induced chromosomal aberrations and mutation

According to MULLER (1935), PAPALASHVILLI has found that the frequency of chromosomal rearrangements in Drosophila is increased by raying the flies at low temperatures. MICKEY (1937) has found a much higher frequency of translocations in flies rayed at 15°C than in those rayed at 28°–34°C. In Tradescantia microspores both chromosome and chromatid aberrations, induced at either the resting or at mid-prophase, are much more frequent in the cells rayed at low temperatures. The variations in temperature affect both one-hit and two-hit aberrations. The decreased frequency of X-ray induced aberrations at the higher temperatures can be attributed to a more rapid fusion of broken ends or to a more relaxed condition of the chromosomes which inhibits chromosome movements. Either factor would favor the reunion of broken ends in the original position and decrease the frequency of illegitimate unions which produce the

aberrations. It has been shown that the frequency of the initial breaks is relatively independent of the temperature.

The reversed temperature effect on the chromatids at earliest prophase is difficult to explain. The reaction of the chromosomes cannot be due to changes in the nucleus as a whole because the single chromosomes show a decreased frequency of aberrations, and the split chromosomes an increased frequency of aberrations at high temperatures. An increase in the speed of union of broken ends may be involved. At earliest prophase the newly formed chromatids may be so closely associated that a break would separate the broken ends of a single chromatid further than the distance between chromatids. At mid-prophase the chromatids may be separated by a distance greater than the distance between broken ends of a chromatid. Rapid union of broken ends would tend to produce lateral fusions and consequently aberrations at early prophase, but would favor reunion in the original position and consequently fewer aberrations at later prophase stages. This interpretation could hardly be applied to the two-hit chromatid aberrations, which also seem to show the reversed temperature effect at early prophase, although the numbers are very low. An increase in torsional strain induced by high temperatures only at earliest prophase could also be invoked to explain these results, but further work is necessary in order to determine the factors involved in the reversed temperature effect at earliest prophase.

The nature of X-ray induced breaks

The chromosomal aberrations induced by X-rays have been attributed to fusions followed by breaks and to independent breaks followed by fusions between broken ends. The contact hypothesis sponsored by MULLER (1932) postulates that the chromosomes in contact or closely associated may be affected by a single X-ray hit. The breakage hypothesis, suggested by STADLER (1931), postulates that independent breaks are followed by illegitimate fusions. The analysis of X-ray induced chromosomal aberrations in Tradescantia confirms STADLER'S hypothesis. The dosage curves for one-hit and two-hit aberrations, the effect of the time-intensity factor, and the temperature effect, all prove that aberrations involving two chromosomes are dependent upon two independent hits.

The one-hit chromatid breaks appear to be caused by single X-ray "hits" since the relation between dosage and frequency of such aberrations is approximately linear. Since two sister chromatids may be broken by a single hit the ionization or excitation must cover a relatively large range because these sister chromatids are separated by a distance of at least a tenth of a micron at mid-prophase. The frequency of induced mutations in Drosophila is independent of X-ray wave-length (TIMOFÉEFF-RESSOV-

SKY 1936). The frequencies of X-ray induced chromosomal aberrations in Tradescantia also are independent of wave-length (RICK, unpublished). These results indicate that the X-ray effects are caused by ionization (GOODSPEED and UBER 1939).

The broken ends of chromosomes may remain in an unstable condition and capable of fusion for an hour or longer. It is surprising that a molecular complex of such a nature can remain in an unstable condition for so long a period. When fusions between broken ends do occur the union appears to be quite perfect in most cases, and breaks in subsequent chromosome bridges are no more likely to occur at the point of union than at other loci. There must be natural breaking points in the chromosome because breaks and fusions are normal features of practically all chromosomes at the time of meiosis. The reunion of both X-ray induced and crossover breaks appears to have no effect on the physical condition of the chromosome.

About five percent of the visible chromosome alterations induced by irradiation of the resting nucleus consist of terminal deletions. The broken ends of such deletions appear to behave as normal ends. Such a behavior may be caused by breaks at loci other than the natural breaking points.

ACKNOWLEDGMENTS

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SUMMARY

An analysis of X-ray induced chromosomal aberrations in the microspores of Tradescantia has led to the following conclusions:

1. The chromosomes react to X-rays as though they were single threads during the resting stage and become effectively split to form sister chromatids at early prophase.

2. The effective splitting of an individual chromosome occurs very rapidly, and there is some evidence that splitting begins at the region of the centromere and progresses distally.

3. The simple terminal deletions are readily induced at prophase because the two sister chromatids may be broken by one hit, followed by lateral fusion of broken ends of sister chromatids.

4. The frequency of these simple deletions shows an approximately linear relation to dosage. The equation for this relation is percent $B = (D/K)^{1.1}$, where B is the number of effective breaks in relation to the total number of chromosomes, D the dosage in r units, and K a constant.

5. A simple deletion induced at the resting stage persists until metaphase only when the broken ends lose their capacity for fusion. Such breaks are rare. The broken ends of these chromosomes appear to behave as normal ends.

6. Aberrations involving two chromosomes, or two loci in the same chromosome, are dependent upon two independent breaks, limited in both time and space.

7. The frequency of these two-hit aberrations is dependent upon the radiation intensity. High intensity is more effective than low intensity because the aberrations are dependent upon two adjacent breaks within certain limits of time. The frequency of one-hit aberrations at a given dosage is independent of the time-intensity factor.

8. The frequency of two-hit aberrations increases exponentially with increased dosage. When the dosage is varied by varying the time of exposure the relation between frequency of aberrations and dosage is percent $B = (D/K)^{1.5}$, but if dosage is varied by varying the intensity the equation is percent $B = (D/K)^{2.0}$.

9. Intermittent dosage experiments show that broken ends of chromosomes may remain in an unstable condition for as long as an hour before fusion, although most fusions occur in a much shorter time.

10. Only a small proportion of the X-ray induced breaks result in visible chromosome aberrations. Most of the broken ends reunite in the original positions with no evident alteration of the chromosome.

11. The frequency of the initial breaks induced by X-rays is relatively independent of temperature. The production of aberrations, however, is greater when the resting and prophase nuclei are irradiated at low temperatures. High temperatures during or immediately following irradiation increase the reunion of broken ends in the original position thus decreasing the frequency of aberrations. This temperature effect is reversed at very early prophase.

12. The initial breaks induced by X-rays must be distributed at random, but both the one-hit and two-hit aberrations occur more frequently at the proximal ends of the chromosome arms. This distribution of effective breaks is attributed to the effect of secondary factors.

13. Differential X-ray production of chromosomal aberrations in different phases of the nuclear cycle, in different cells of the same organism, and in similar cells of different organisms, can be attributed, in part, to secondary factors, such as proximity of chromosomes, freedom of chromosome movement, and the physical condition of the individual chromosome.

14. The X-ray induced breaks appear to occur usually at natural breaking points. Most of the breaks and fusions following irradiation appear to be comparable to those occurring naturally at the time of crossing over.

ADDENDUM

I have seen, in manuscript, a paper on X-rayed Tradescantia chromosomes submitted to "Genetics" in July 1939 by A. C. FABERGÉ of University College, London. FABERGÉ's conclusions on the time factor and the temperature effect are essentially the same as those reported above. His data were not adapted to an analysis of the dosage curve, and his consions in this regard appeared to require reconsideration.*

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SPONTANEOUS CHROMOSOME ABERRATIONS IN TRADESCANTIA

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THE occurrence of spontaneous chromosome aberrations has been observed in numerous genera of both plants and animals. These natural structural changes have consisted largely of reciprocal translocations and inversions, which when heterozygous result in characteristic configurations visible at meiosis. Similarly, such changes are evident in the salivary gland chromosomes of Drosophila. They also may be detected by genetic analysis where the linkage maps are adequate. Structural alterations of this nature may persist indefinitely, since they are usually viable in the haploid stage of the chromosome cycle. Many other types of structural changes must occur only to be lost because of lethal deficiencies. Spontaneous chromosome alterations are usually so rare that no adequate analysis can be made of the types and the frequency of their appearance. Further, it is seldom possible to determine the stage in the nuclear cycle at which the aberration occurred. In Tradescantia, however, a number of plants have shown a comparatively high frequency of spontaneous chromosome alterations, and the types of alterations found in the microspore chromosomes indicate their time of origin in the nuclear cycle.

In this analysis we have used several pure species of Tradescantia and the F_1 and F_2 plants from a natural species hybrid between *T. canaliculata* Raf. $\times$ *T. humilis* Rose. Smear preparations were made of the developing microspores at the first post-meiotic mitosis. The chromosomes were analysed for types and frequencies of natural chromosome aberrations. The nature of the spontaneous alterations suggested that natural radiation might be involved, and for comparative purposes the alterations induced by gamma rays and X-rays have been analysed.

TYPES AND FREQUENCIES OF X-RAY INDUCED AND SPONTANEOUS CHROMOSOME ABERRATIONS

The effects of X-rays on the chromosomes in the developing microspore of Tradescantia have been studied in considerable detail by several investigators (RILEY 1936; HUSTED 1936; MATHER 1937; SAX 1938; SAX and MATHER 1939). Their results have shown that the configurations produced by irradiation comprise two distinct classes, depending on whether the breaks and subsequent fusions occur before or after the effective splitting of the chromosomes. Breaks occurring when the chromosomes are

split are termed chromatid breaks, those when they are single, chromosome breaks. In the case of both classes, the breaks may involve only a single chromosome or may result in exchanges between two or more chromosomes. A study of the relation between types of breaks and dosage (SAX 1940) has shown that some configurations result from a single X-ray "hit" and some from two "hits." A single break involving only one of the two sister chromatids gives a shortened chromatid arm and an acentric fragment at the microspore division. If the hit breaks both arms, adjacent ends of sister chromatids fuse, and a dicentric chromatid and a U-shaped acentric fragment results. Chromatid exchanges involving two non-homologous chromosomes depend on two independent breaks. The types of chromosome breaks, which occur as a result of irradiation during the microspore resting stage, show similar relations to dosage. A single break in one chromosome results in a deletion; when the chromosome later divides the broken ends do not fuse—in sharp contrast to the behavior of ends of sister chromatids broken at the same locus—but give two shortened chromosomes and two rod-shaped acentric fragments at metaphase. Two breaks, one in each arm of the same chromosome, may reunite to form a ring chromosome. At anaphase, after splitting, separation of the ring may be free, interlocked, or as a single double size dicentric ring. Chromosome exchanges, involving two non-homologous chromosomes, also depend on two X-rays hits. Reunion of ends results either in a reciprocal interchange, which is usually equal in the case of Tradescantia, and hence not detectable, or in a dicentric chromosome and an acentric fragment. The dicentric divides normally, and at anaphase may separate freely, form two bridges, or interlock, depending on the amount of relational coiling between the centromeres. The fragment also divides normally, and produces two rod-shaped acentric fragments at metaphase.

A study of the relation between the frequency of the various types of chromosome and chromatid breaks and the X-ray dosage has led to the derivation of specific equations relating the percentage of breaks to the dosage in roentgens (SAX 1940). For one-hit chromatid breaks this equation is $B = (D/45)^{1.1}$, where B = percent breaks and D = dosage in roentgens (r). The single breaks evidently tend to increase directly with the dosage. For the two-hit chromatid exchange breaks, however, the equation is $B = (D/67)^{1.9}$, the increase in breaks being approximately as the square of the dosage. A comparison of actual counts on which these equations are based shows that the one-hit breaks are much more frequent at low dosages than the two-hit. For example, at 10r the frequency of one-hit chromatid breaks is .2 percent whereas that of the two-hit breaks is .03 percent. At 100r, however the frequencies are almost equal, the one-hit breaks equal 2.4 percent, and the two-hit 2.0 percent. The percentage of

dicentric and ring chromosomes at 10r is .01 percent, and at 100r is .8 percent.

A study of the position in the chromosome of X-ray induced breaks (SAX and MATHER 1939) has shown that they do not occur at random, but tend to be localized in the regions proximal to the centromere.

The types of chromosome aberrations found occurring spontaneously are very similar to those produced by irradiation. The most frequent is the chromatid break involving both sister chromatids at the same locus, and resulting in a dicentric chromatid bridge accompanied by a fused acentric fragment at anaphase (Plate I, figure A, B, C). Single chromatid deletions also occur, and one case of a chromatid exchange, producing a dicentric chromatid and an acentric fragment, was observed in a triploid (figure H). Chromsome breaks were observed less frequently than chromatid, and single chromosome deletions (figure F) are more common than exchange breaks involving two chromosomes. Aberrations which had occurred previous to or at the time of meiosis were noted occasionally. In this category at metaphase are included those chromosomes which have lost all or part of an arm and have no accompanying acentric fragment. They have been observed only in microspores containing at least a normal haploid complement. It is probable that the centric fragments which occur with considerable frequency in certain species of Tradescantia (WHITAKER 1936) are the result of such losses (figure G). A few unequal reciprocal translocations which had evidently occurred during the post-meiotic stages were also noted.

The chromosome configurations resulting from spontaneous structural changes during the post-meiotic development of the microspore must be carefully distinguished from somewhat similar types which may appear at the microspore mitosis as a result of chromosome behavior at meiosis. There is no difficulty in distinguishing single chromosome and chromatid breaks, producing shortened arms and acentric fragments, for these could only result from single breaks with no subsequent fusion of ends during the post-meiotic development of the microspore. In the case of bridge configurations, however, more care is necessary.

The principal types of chromosome bridge configurations which may occur at the first post-meiotic mitosis in pollen grains and their methods of origin are represented in figure 1. Most of these types have been recorded as occurring spontaneously or as a result of irradiation, and although some other types are also theoretically possible according to our present knowledge of chromosome behavior, they must be of extremely rare occurrence.

Dicentric chromosomes and chromosome bridges may result from chromosome behavior at meiosis or during the post meiotic development of the microspore. Crossing over in heterozygous inversions at meiosis may lead

to two distinct types of dicentrics at the succeeding pollen grain mitosis. Non-disjunction at second anaphase of a dicentric chromatid resulting from single crossing over within an inversion and disparate crossing over proximal to the inversion produces a dicentric chromosome which divides

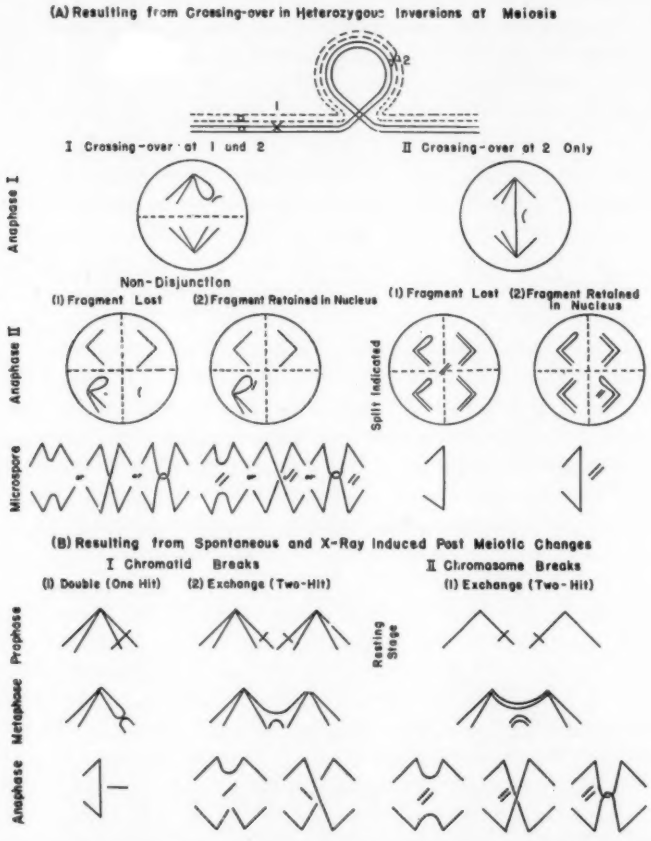

FIGURE 1.—Principal types and methods of origin of chromosome bridge configurations occurring at the first post-meiotic mitosis in microspores. (For discussion see text.)

mitotically and separates at anaphase of the pollen grain mitosis (figure 1; A, 1). The manner of separation depends on the amount of relational coiling between the centromeres, and may be free, criss-cross, or interlocked. This non-disjunction type has been noted in Rhoeo by DERMEN (EMSWELLER and JONES 1938), in a triploid Tulipa by UPCOTT (1937), and in a triploid Tradescantia (GILES, unpublished). Normally the resulting

acentric fragment is not included in the nucleus, but if included through both divisions it will divide and appear as two acentric rods at metaphase. The suggestion has been made by EMSWELLER and JONES (1938) that such bridge configurations may also result, particularly in hybrids, from the distribution of dicentrics formed as a result of crossing over in the region between the centromeres of chromosomes paired in such a manner that their centromeres are not opposite each other. The second type of dicentric produced at the microspore mitosis results from the behavior of broken ends of chromatids following the breakage of an inversion bridge due to a single crossover within an inversion (figure 1; A, II). In this case, the broken ends of the inversion bridge either fail to divide normally during the succeeding resting stage, or, if the chromosome is considered as already split, the ends of sister-half chromatids fuse. The result in the case of either interpretation is a dicentric chromatid. The resulting single bridge of this type was described by SAX (1937) in a triploid Tradescantia, and in Zea by MCCLINTOCK (1938). In many plants the acentric fragment produced by the inversion crossover is lost in the cytoplasm during meiosis. In Zea however, it is sometimes included within the nucleus through both meiotic divisions, and in such cases, after division during the resting stage, two rod-shaped fragments are visible at the time of the pollen grain mitosis.

The types of bridges resulting from aberrations occurring during the post-meiotic development of the microspore have already been described. The single chromatid dicentrics produced by breakage and fusion between sister chromatids at the same locus are accompanied by single acentric fragments which distinguish them from those cases in which the acentric fragment is included in the nucleus with a broken bridge at meiosis. Single dicentrics resulting from chromatid exchanges may pass to one pole or form a bridge; single acentric fragments are present in these cases also (figure 1; B, 1). Chromosome exchanges produce dicentrics which give the same configurations as do the meiotic non-disjunction dicentrics. In this type, however, two rod-shaped acentric fragments are normally always present (figure 1; B, II).

The occurrence of single chromatid bridges at anaphase of the microspore division in a triploid Tradescantia and a diploid Hyacinthus has been noted by UPCOTT (1937) and attributed to post-meiotic spontaneous structural changes in the chromosomes. No distinction is made, however, between the types of bridges noted. As in the case of the Tradescantia, those bridges accompanied by single acentric fragments were evidently due to the changes inferred. The bridges without fragments, however, presumably resulted from the breakage of inversion bridges at meiosis. In the Hyacinthus, few fragments were observed, and since the bridges appeared to be about equal in length to the sum of the two free arms—the

chromosome involved had a median centromere—the conclusion is drawn that normal chromosome ends have fused. This observation does not agree with most of the recent X-ray evidence, which has supported the idea that normal ends have properties not possessed by interstitial loci. It seems much more probable that such bridges without fragments are also the result of breakage of inversion bridges at meiosis. The length of the bridge could be accounted for if the inversion were very near the end of the chromosome. Such a condition would also favor the occurrence of bridges at the microspore division, for the small deletion resulting from the crossover would quite possibly not be sufficient to prevent the post-meiotic development.

Observations on the occurrence of spontaneous chromosome aberrations observable at the pollen tube mitosis in Paeonia and Kniphofia by BARBER (1938) are also interpreted as demonstrating the fusion of homologous ends of sister chromatids. Although no particular study was made at this stage in Tradescantia, the configurations observed lend no support to the above conclusion. Only bridges with acentric fragments were noted (Plate I, E). The pollen used was not subjected to controlled aging, however, as was the case in the study cited. It is possible that aging of pollen may produce results similar to the initial effects of heat and X-rays which cause terminal fusions, apparently involving the chromosome envelope (SAX 1938).

In the diploid plants of Tradescantia examined in the present study no bridges at the microspore mitosis without fragments were noted. The absence of single bridges without fragments apparently shows that even small deletions are sufficiently lethal in the haploid condition to prevent the development of the microspore. It is possible, however, that further

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DESCRIPTION OF PLATE I

Photographs of aceto-carmine smear preparations showing spontaneous chromosome aberrations in Tradescantia. Ca. 1200×.

FIGURE A.—Microspore anaphase in diploid. Dicentric chromatid and fused acentric fragment, upper right.

FIGURE B.—Microspore anaphase in diploid. Dicentric chromatid and fused acentric fragment at left.

FIGURE C.—Microspore anaphase in diploid showing seven chromosomes as a result of nondisjunction. Stretched bridge of dicentric chromatid, with long acentric fragment above.

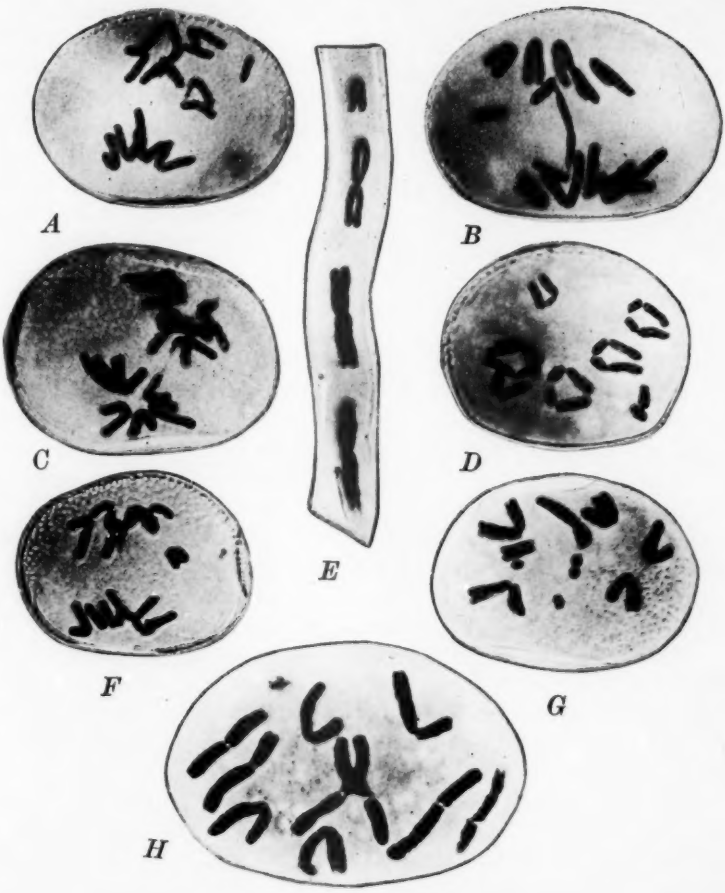
FIGURE D.—Microspore early anaphase in diploid. Dicentric chromatid. Two acentric fragments at lower right, apparently resulting from failure of fusion of ends.

FIGURE E.—Pollen-tube division in diploid, only three chromosomes shown. Dicentric chromatid and U-shaped acentric fragment at top.

FIGURE F.—Microspore anaphase in diploid. Single chromosome break showing divided acentric fragments. Shortened chromosomes at poles.

FIGURE G.—Microspore metaphase in diploid. Extra centric arm and four centric fragments present.

FIGURE H.—Microspore metaphase in triploid. Chromatid exchange break.


A
B
C
D
E
F
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H

study will reveal that this is not always the case. In only one case was a chromatid bridge with two fragments observed (Plate I, D). A study of this figure indicates that it is probably due to the failure of fusion of the distal ends of sister chromatids following a double chromatid break. Similar behavior has been noted very rarely in the case of X-ray induced double chromatid breaks. Observations at meiosis indicate that acentric fragments resulting from inversion crossovers are not included in the nucleus in Tradescantia, and the almost complete absence of single bridges with divided fragments substantiates this. In triploids, single bridges both with and without single fragments have been found, but no cases of bridges with double fragments have been seen, again indicating that fragments at meiosis are usually lost in the cytoplasm.

In all the cases of chromosome dicentrics observed, two rod-shaped fragments were plainly visible. Although it is theoretically possible, as indicated in figure 1, that such configurations might be the result of nondisjunction of a dicentric chromosome at meiosis with the fragment being included in the nucleus, this is highly improbable. All the evidence indicates that a fragment is normally lost in Tradescantia and the chances of its being retained in the nucleus with the dicentric through both divisions at meiosis, as would be necessary to give a configuration similar to those observed, is negligible.

There is good evidence from recent X-ray experiments designed to determine the time of chromosome division that splitting occurs during the early prophase preceding the first post-meiotic mitosis in the microspore (MATHER 1937; SAX and MATHER 1939). Although it is not possible to determine the time of occurrence previous to the microspore mitosis of the various types of spontaneous aberrations, the fact that both chromatid and chromosome breaks occur and are not the result of natural radiation, as will be shown later, supports the X-ray evidence. It indicates that the kind of breakage resulting from irradiation is not due to any peculiar effect of the ionization itself, as has been suggested. However, the behavior of broken ends of chromatid bridges broken at meiosis appears not to support the X-ray evidence, for the resulting bridge formation can be most easily understood if the chromatid is considered to be already divided at meiosis. On the other hand, if the chromosome entering the resting stage is considered to be already split, the types of spontaneous breaks and the behavior of X-ray induced breaks are not easily explained. It seems possible that the behavior of the ends following bridge breakage may not be the same as in the case of X-ray induced and spontaneous post-meiotic breaks. The bridge chromosome apparently breaks under tension, or perhaps as a result of cytoplasmic enzyme action as suggested by EMSWELLER and JONES (1938), and it is possible that the resulting

damage at the locus of breaking may inhibit the later splitting of the chromosome at the region of the newly formed ends. Such a failure of division at the ends would result in a single dicentric bridge at the following mitosis. Single dicentric bridges without fragments would also result if the broken ends of meiotic bridges retained their ability to fuse until after splitting had occurred. The X-ray evidence indicates, however, that broken ends retain this ability for only about an hour after breakage (SAX 1939).

The single chromosome deletions, as well as the chromatid deletions, are of particular interest since they indicate that new stable ends may arise as a result of spontaneous breaks. Most of the evidence on chromosome aberrations shows that broken ends tend to fuse, and it has been suggested that permanent changes in the chromosome morphology cannot be effected

TABLE 1

Loci of spontaneous chromatid and chromosome breaks. Each locus includes one-fifth of chromosome arm, starting with locus one proximal to centromere and extending distally to end of arm.

LOCUS	CHROMATID		CHROMOSOME	
	N	%	N	%
1	24	50	10	36
2	7	15	11	39
3	8	17	5	18
4	5	10	2	7
5	4	8	0	0
Total	48		28	
Ave. Locus	2.0		1.1	

by terminal deletions. However, it now seems clear that stable ends may arise with a fair frequency as a result of both X-ray induced and spontaneous terminal deletions.

A study of the loci of the spontaneous breaks with respect to the centromere was made. To do this the various arms were divided into five loci measuring from the centromere to the ends. The relative positions of the breaks rather than the actual distance were used since the arms vary somewhat in length, and the individual chromosomes cannot be differentiated. The data are presented in table 1. It is evident that, as in the case of the X-ray induced breaks, the distribution of the observed aberrations is not at random. For chromatid breaks the average locus of the breaks is 2.0, and for chromosome breaks it is 1.1. This indicates a distinct localization in the region proximal to the centromere.

The relative frequencies of the different kinds of aberrations occurring spontaneously are also very similar to those obtained from irradiation at low dosages. The results of the present study are indicated in the totals to table 2. In the 78,021 chromosomes examined to determine the fre-

quency of the various types of alterations, there were 55 chromatid breaks giving dicentric chromatids, 26 chromosome deletions, and 2 dicentric chromosomes. Dicentric chromatids are therefore about twice as frequent

TABLE 2

Frequency of various types of spontaneous chromosome aberrations. Examples from hybrid F_2 to indicate variation. Totals of all plant groups.

PLANT	DATE	TOTAL CHROMOSOMES	ONE-HIT				TWO-HIT			
			DICENTRIC CHROMATIDS	%	CHROMOSOME DELETIONS	%	DICENTRIC CHROMOSOMES	%	TOTAL BREAKS	%
F_1										
Gentilly 5		2868								
Gentilly 13		1434	2	.14					2	.14
F_2 125	2/27/39	1956	4	.20	2	.10			6	.30
	3/ 3	1392								
	3/20	1260								
	5/ 8	1146	2	.17					2	.17
346	11/ 5/38	1290			1	.08			1	.08
	11/ 6	1326	1	.07					1	.07
	11/ 7	1327*								
	11/ 9	1296								
	11/10	1290								
	11/11	1262								
93	2/21/39	762	2	.26					2	.26
	2/26	1362	2	.15					2	.15
	3/ 1	1344	3	.22					3	.22
	3/ 1	546	1	.18					1	.18
	3/22	816			2	.24			2	.24
35	10/ 6/38	1821	4	.22	2	.11			6	.33
	2/15/39	1248	1	.08	2	.16			3	.24
	2/18	1260	1	.08					1	.08
	3/ 5	1362	4	.30					4	.30
Total F_2 (21 plants)		70,447	54	.076	26	.037	1	.001	81	.12
Total non-hybrid plants		7,574	1	.02	—	—	1	.02	2	.04
Total all plants		78,021	55	.070	26	.033	2	.003	83	.11

* Totals not multiples of six indicate occurrence of microspores with seven chromosomes as a result of non-disjunction at meiosis.

as chromosome deletions, and about thirty times as frequent as dicentric chromosomes. It will be recalled that at low irradiation dosages, one-hit chromatid breaks giving dicentric chromatids are much more frequent

than both chromatid and chromosome two-hit breaks. The ratio of single chromatid breaks to chromatid exchange breaks is of the order of 10 to 1; that of single chromatid to chromosome exchanges is about 20 to 1. Although there is no very adequate X-ray data for the one-hit chromosome deletions, the frequency of these types is evidently very much lower than that of the one-hit chromatid breaks involving both chromatids at the same locus. This is to be expected, as it seems clear that many more breaks occur as a result of irradiation than appear as visible aberrations following fusions in new associations. Single breaks occurring when the chromosome is single are very likely to heal back in the same position, and are thus not detected. Only a few fail to heal and lead to visible deletions. When a single hit breaks both chromatids of a chromosome at the same locus after division, however, the subsequent fusion of the adjacent broken ends produces a dicentric chromatid and a U-shaped acentric fragment, and the aberration is detected at the succeeding division.

The comparative frequencies of single chromosome deletions and one-hit chromatid breaks indicate that the former occur with a considerably greater relative frequency spontaneously than when X-ray induced. This seems to be due to the fact that the period during which chromosome deletions may occur lasts for from eight to ten days whereas that during which single chromatid breaks may originate is approximately 24 hours in length.

Single breaks occurring in one chromatid of a chromosome were not included in the tabulation since their frequency is extremely difficult to record accurately. As in the case of observations following irradiation, the degree of breakage may vary from achromatic lesions through partially broken ends to complete deletions.

SPONTANEOUS CHROMOSOME ABERRATIONS AND NATURAL RADIATION

The similarity between the types, the loci, and the frequency of chromosome aberrations occurring spontaneously and those produced by irradiation at low dosages suggested the possibility that the former might be caused by natural radiation. If this is the case, increasing the amount of irradiation above that to which the plants are normally subjected due to natural radiation (taken as 50 ions/cc/sec = .0022 r/day) should result in an increased frequency of breaks. In order to make this test at the low dosages involved, a radium capsule of known ionizing strength (5.4×10^{-6} r/min at 50 cm) was used as a gamma ray source. By placing this at various distances from the plants in accord with the inverse square law, it was possible to vary the irradiation intensity as compared to natural radiation. Two plants were tested, #393, which had shown a fairly constant frequency of spontaneous aberrations (table 2) and #404, in which

no aberrations had been noted. Double chromatid breaks, which are classed as one-hit chromatid breaks and result in a single bridge and an acentric fragment at anaphase, were used in the calculations for several reasons. They occur more frequently than any other type, and hence a better measure of their frequency can be obtained. Then, too, the period during which this type of break may originate is accurately known from the X-ray evidence, and consequently the amount of natural radiation to which the plant is subjected can be ascertained. Chromatid breaks are observed in the microspore within five hours after irradiation, and reach a distinct frequency peak at about 24 hours. The frequency then declines rather rapidly, and no chromatid breaks are found after 70 hours during the winter months. The average period during which the majority of breaks occur seems to be approximately 24 hours in length. It is also better to use one-

TABLE 3

Test by use of radium capsule of rôle of natural radiation in the production of spontaneous chromosome aberrations.

DISTANCE OF CAPSULE (CM)	TIME	APPROXIMATE AMOUNT BY WHICH NATURAL RADIATION IS INCREASED	#393			#404		
			TOTAL CHROMOSOMES	DICENTRIC CHROMATIDS (ONE-HIT)	%	TOTAL CHROMOSOMES	DICENTRIC CHROMATIDS (ONE-HIT)	%
30	24 hrs.	10	2329	3	.13	1254	—	—
3	24 hrs.	1000	1344	3	.22	1350	—	—
Control			1578	4	.26	1344	—	—

hit chromatid breaks rather than exchange breaks since it has been shown by SAX (1939) that this type of break is independent of the time factor. Consequently, even though they may occur at any time during the 24 hour period, their spontaneous frequency may be compared with the frequency of similar breaks induced by irradiation of known dosage acting for a short or long interval. The value used to represent the intensity of natural radiation, 50 ions/cc/sec, is that given by TIMOFÉEFF-RESSOVSKY (1931). The data are presented in table 3.

The amount of ionization produced by the capsule at a distance of 3 cm over a 24 hour period is approximately 2.2 r. Although this intensity would be expected to produce only about .05 percent aberrations, it is 1000 times that due to natural radiation. Even with this great increase in intensity, there was no increase in the number of aberrations.

Further evidence that natural radiation is not sufficient to account for the observed frequency of spontaneous breaks is obtained by comparing the actual frequency of these breaks with the expected frequency if ioniza-

tion due to natural radiation causes the breaks. As has been noted, the chromatid breaks studied depend on one X-ray hit and show an approximately linear increase with the dosage. For Tradescantia the equation relating the frequency of breaks to the dosage in roentgens is $B = (D/45)^{1.1}$. Using this equation, the percentage of breaks produced by a given X-ray dosage (for example, 100 r) and that which would be produced by a dosage equal to the intensity of natural radiation can be calculated and compared. The same value as before is used to represent the intensity of natural radiation. The frequency of one-hit spontaneous aberrations, .07 percent, is the percentage of breaks recorded in the total of 78,021 chromosomes of both hybrid and non-hybrid plants examined (table 3). The calculations are outlined as follows:

1. Equation for one-hit (double chromatid breaks): percent B = (Dosage in r/45).$^{1.1}$
2. Percent B at 100 r = 2.4 percent.
3. Amount of natural radiation = 50 ions/cc/sec.
4. Period during which double chromatid breaks occur = 24 hours = 8.6×10^4 sec.
5. 1 r = 2×10^9 ions/cc.
6. Amount of natural radiation occurring during the 24 hour period = $\frac{50 \times 8.6 \times 10^4}{2 \times 10^9} = .0022$r.
7. Percent of one-hit breaks occurring spontaneously = .07.
8. Amount of radiation necessary to produce .07 percent B = 4.0 r.
9. ∴ 4.0 r/.0022r = ca 1818—the amount by which the natural radiation intensity is too small to account for the observed spontaneous break frequency.

The value given by TIMOFÉEFF-RESSOVSKY, 50 ions/cc/sec, and used in the present calculations for the amount of ionization due to natural radiation probably represents a maximum. MULLER and MOTT-SMITH (1930) used 30, and BLACKETT (1935) gives the value as between 20 and 30 ions/cc/sec. The use of the higher value tends to keep the result indicative of the amount by which the intensity of natural radiation is too small to account for the rate of spontaneous chromosome aberrations on the conservative side. However, it is also possible that a longer period should be allowed for the action of natural radiation in the production of chromatid breaks during the period preceding the microspore division. This would tend to lower the calculated value.

The actual frequency of all one-hit spontaneous breaks occurring during the 24 hour period is undoubtedly higher than .07 percent. Single chromatid deletions were not recorded, and neither inversions, unless they involve

a considerable change in the position of the centromere, nor small ring or rod deletions can be detected at the microspore metaphase. It has been shown by HUSTED (1937) that structural changes may occur with some frequency during the development of the microspore of *Paris quadrifolia* and *Pancratium illyricum* without abnormalities being found at metaphase or anaphase. It is perfectly legitimate to use this percentage in the preceding calculations, however, since the X-ray equation in which it is used is also based on counts of one-hit chromatid dicentrics only.

Since one of the plants tested with the radium capsule, #393, showed a rather high and fairly constant rate of spontaneous chromosome alterations while the other, #404 showed none at all, it was decided to test the sensitivity of the two plants to the same dosage of X-rays. It might be expected *a priori* that the plant showing the higher percentage of spontaneous breaks would have a lower threshold of response and be more sensitive to X-rays, especially if natural radiation were the cause of the

TABLE 4

Sensitivity to X-rays. 320 r. Examined for chromosome breaks five days after raying.

PLANT NO.	TOTAL CHROMOSOMES	NO. OF BREAKS	PERCENT
393	4062	408	10.4
404	4566	502	10.9

breaks. Inflorescences of both plants were rayed at the same time and received a dosage of 320 r (80 r/min for 4 min). The buds were examined after five days for chromosome breaks. Three separate tests were made at different times, and the combined data are presented in table 4.

The results show that the two plants are equally sensitive to the same dosage of X-rays and may be taken as further support for the conclusion that natural radiation is very rarely involved in the production of spontaneous chromosome aberrations. The failure to find an increased sensitivity to X-rays in the plant showing a high frequency of spontaneous chromosomal aberrations is somewhat similar to the effect of irradiation on the rate of change in unstable genes of *Drosophila virilis* (DEMEREC 1934). In the latter case, however, an X-ray dosage which would increase the general mutation rate by about one thousand percent did not produce a significant increase in the rate of change of the unstable gene tested. In the present study, although there was no differential sensitivity, the rate of chromosome aberrations was increased by the X-ray dosage by about the expected amount. This is to be expected since the chromosome aberrations are not reversible reactions as has been postulated to be the case for the unstable gene mutations.

The evidence that the rate of spontaneous chromosome aberrations in Tradescantia cannot be accounted for in terms of natural radiation agrees with the results obtained for natural mutations in Drosophila. When it was first demonstrated by MULLER that irradiation produces a marked increase in the mutation frequency in Drosophila, it was at once suggested that natural radiation might be the cause of spontaneous mutations. Early discussions and experiments by OLSON and LEWIS (1928), BABCOCK and COLLINS (1929), and HANSON and HEYS (1930) appeared to indicate that this might be the case. It was soon shown, however, by the calculations and experiments of MULLER and MOTT-SMITH (1930) and TIMOFÉ-EFF-RESSOVSKY (1931) that the amount of ionization due to natural radioactive substances is much too small to account for the natural mutation frequency. The results obtained by MULLER and MOTT-SMITH indicated that the natural mutation frequency in Drosophila is at least 1300 times as high as it would be if it were caused solely by natural radiation; those of TIMOFÉEFF-RESSOVSKY, that it was at least 500 times as high. Later, the possible rôle of cosmic rays in causing mutations was suggested by THOMAS (1936) and others. But in this case, too, it has been fairly clearly shown by the calculations of DELBRÜCK and TIMOFÉEFF-RESSOVSKY (1936) and the experiments of JOLLOS (1937, 1939) that the amount of this type of ionizing radiations is also much too small to account for the natural mutation frequency.

NATURE OF SPONTANEOUS CHROMOSOME ABERRATIONS

It has been noted that the localization of spontaneous breaks in the proximal region of the chromosomes agrees with the findings in the case of X-ray induced breaks (SAX 1938; SAX and MATHER 1939). As pointed out in the latter paper "the initial breaks induced by X-rays should be distributed at random. There is good evidence that most of the broken ends reunite in the original association and that the permanent aberrations are associated with tortional strains which inhibit normal reunion of broken ends of chromatids and chromosomes . . . . The localization of breakage is attributed to the existence of a greater tortional stress in the proximal regions." In the case of the spontaneous aberrations, however, there is no obvious reason to assume a random distribution of breaks. Consequently, the localization of the breakage in these cases suggests that the tortional strains may be directly responsible for the breaks, not secondary factors as in the case of the X-ray induced aberrations. The much higher frequency of alterations occurring after the chromosomes are split into chromatids than when they are unsplit appears to be correlated with the difference in the nature and amount of coiling at these two stages. During the resting stage only relic coiling is present, whereas during the prophase

the sister chromatids are relationally coiled as well. The X-ray evidence also supports this conclusion since sensitivity of the chromosomes, as measured by the frequency of breaks reuniting in new associations, is greater during the prophase, when both types of coiling are present, than during the resting stage. It has been demonstrated that seedlings grown from aged seeds show a pronounced increase in the frequency of spontaneous chromosome structural changes (NAVASHIN 1933, NAVASHIN and GERASSIMOVA 1936, PETO 1933). As has been pointed out, this evidence suggests that the physiological conditions attendant on aging may be the cause of the increase in chromosome alterations. If chromosome structural mutations in general are the result of physiological conditions within the cell, not affecting the chromosome coiling mechanism, the loci of such breaks might be at random. In such a case the localization of the breakage in the regions proximal to the centromere would then be considered a secondary effect, as in the case of X-ray induced breaks.

The frequency of spontaneous structural changes of the types studied varied with the individual plants used. Also, some plants were found that showed a fairly constant percentage of aberrations whenever examined, while others varied considerably from one observation to the next. Some selected examples, presented in table 2, illustrate these points. There was no evidence of unusual meiotic behavior in individuals of the hybrid population showing a constant and high frequency of chromosome aberrations at the microspore mitosis. The chiasma frequency, percent of inversion bridges, and chromosome distribution were approximately the same as in the plants showing few or no spontaneous structural changes. There is some indication, however, that the difference between the plants of the F_2 generation may have a genetic basis. One of the F_1 parents showed no breaks in 2868 chromosomes examined, while the other showed two breaks in 1434 chromosomes, or .14 percent. The percentage of breaks in the F_2 segregates varied considerably. Some plants were found with frequencies considerably higher than the average for the F_2 of .12 percent. The highest frequency recorded was .48 percent. Other plants had a very low frequency, some showing no breaks in any of the chromosomes examined. Similar evidence that spontaneous structural chromosome changes may be genetically controlled is indicated by the "sticky chromosome" mutant of Zea (BEADLE 1932) and the variation in frequency of aberrations in the F_1 progeny of X-ray treated plants of *Crepis capillaris* (LEVITSKIJ 1937). The presence of genetic factors affecting the frequency of both visible and lethal spontaneous gene mutations in *Drosophila melanogaster* has been reported by DEMEREC (1937). Here, however, the genetic tests indicated that the spontaneous lethals were not connected with chromosomal aberrations.

The frequency of spontaneous breaks in the hybrid plants was about three times as high as in the pure species examined. It is well known that hybridization often results in irregularities in chromosome behavior which may produce changes in the number and morphology of the chromosomes (NAVASHIN 1927, 1934, MÜNTZING 1934, EMSWELLER and JONES 1938). These changes are usually associated with the mode of pairing and crossing over at meiosis in the hybrid, but there is also evidence for their spontaneous occurrence in somatic cells. In the case of some Triticum-Secale hybrids (PLOTNIKOWA 1932) the frequent occurrence of chromatid bridges and fragments at anaphase in root-tip mitoses was noted. Here, too, there was considerable variation in the frequency of bridge formation among the individuals of the same filial generations. It seems possible that the genetic recombination resulting from hybridization may be the cause of the higher percentage of visible structural chromosome aberrations, perhaps by disturbing the normal timing of the cycle of chromosomal coiling.

The occurrence of a high frequency of spontaneous chromosome aberrations in hybrids may perhaps be of significance in species formation, as it now seems clear from numerous studies that specific differences in both plants and animals are quite often associated with chromosome rearrangements. In the genus Tradescantia, hybridization under natural conditions is quite frequent (ANDERSON 1936, 1938) and this should provide opportunity for an increased number of structural chromosome changes.

Recently, a high mutation frequency has been noted in hybrids between races A and B of *Drosophila pseudoobscura* by STURTEVANT (1939). It is stated that there is no indication of an increase in major chromosome aberrations such as accompany X-ray treatment. Small rearrangements have not been investigated, however. It is still quite possible that chromosome aberrations of the type reported in the present Tradescantia hybrids may occur with some frequency in the Drosophila hybrid. Single chromatid dicentrics, which constitute the majority of the breaks found in Tradescantia, would not be viable, and the present method fails to indicate small inversions and deletions. These latter aberrations apparently show a linear increase with X-ray dosage, as do mutations, and such aberrations may be the cause of many if not most of the visible and lethal mutations. Evidence of a correlation between the occurrence of mutations and chromosome rearrangements is indicated by the work on Sciara by METZ and BOCHE (1939). In the progeny of X-rayed virgin female flies there is a very low incidence of both mutations and chromosome rearrangements. On the other hand, a high frequency of both rearrangements and mutations occurs as a result of X-raying sperm. The evidence from the present study that natural radiation is insufficient to account for the frequency

of spontaneous chromosome aberrations establishes a further similarity between mutations and chromosome aberrations.

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SUMMARY

The types, frequency, and loci of spontaneous structural chromosome aberrations in a number of pure species and in the F_2 generation of a natural species hybrid of Tradescantia have been studied at the first post-meiotic mitosis. These aberrations are similar in most respects to chromosome alterations induced by low dosage X-rays in the developing microspore. This similarity suggested that the spontaneous breaks might be due to natural radiation. Considerable evidence is presented, however, to show that this is not the case. By employing a radium capsule, the plants were subjected to irradiation 1000 times that due to natural radiation, but no increase in aberrations was found. Using X-ray data relating percentage of breaks to dosage, the dosage required to account for the observed frequency of spontaneous breaks was calculated. The amount of natural radiation was found to be approximately 1800 times too small to account for this frequency. Plants showing a considerable difference in the percentage of spontaneous breaks showed no differential sensitivity to the same X-ray dosage.

The localization of the breaks in the region of the chromosome arms proximal to the centromere, and the greater frequency of chromatid as compared with chromosome breaks suggests that the tortional strains associated with relic and relational coiling during the chromosome cycle may be a major factor in the causation of spontaneous breaks.

The frequency of spontaneous aberrations was found to vary widely among the individuals of the F_2 generation of the hybrid, and evidence is presented suggesting the genotypic control of spontaneous chromosome structural aberrations.

The average percentage of breaks in the hybrids was about three times that of the pure species. It is suggested that this increase in aberrations may result from the recombination of genetic factors following hybridization.

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THE INHERITANCE OF INTERGENERIC DIFFERENCES IN ZEA-EUCHLAENA HYBRIDS[1]

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INTRODUCTION

IN THIS study of Zea-Euchlaena hybrids and their segregating populations, all those characters (such as width of leaves and number of tillers) which show wide variation in both maize and teosinte were ignored and only characteristic differences were considered. Large populations of F_2 and backcross plants were grown to study the segregation of weak versus strong response to length of day, paired versus single female spikelets, many-ranked versus two-ranked ear, and many-ranked versus two-ranked central branch of the tassel. A brief abstract of this work has been published (LANGHAM 1939).

PHOTOPERIODISM

Weak versus strong response to length of day

The extensive research of GARNER and ALLARD (1920, 1923) has shown that length of day is of primary importance in determining the time of flowering of plants. Reproductive processes are initiated in some plants, such as red clover, iris, goldenrod and spinach by a relatively long day, while other plants, such as cosmos, soybean, violet and poinsettia require a relatively short day for flowering and fruiting.

EMERSON (1924) has shown that Durango teosinte, *Euchlaena mexicana*, is a member of the latter group; it is a short-day plant. If grown under long-day conditions it will remain in the vegetative state throughout the year. It can be forced into flowering in any season of the year, however, by exposing it for 30 days to short-day conditions (13 hours or less of daylight in each 24-hour period). If planted in Ithaca about May 15th, Durango teosinte will shed pollen early in October, because the 13-hour day begins about September 1st.

Maize of medium maturity planted in Ithaca about May 15th sheds pollen the first part of August. This difference of two months (August to October) in the flowering habits of maize and teosinte, and the fact that the hybrid between the two genera is highly fertile, suggested the possibility of studying the mode of inheritance of photoperiodism in maize-teosinte hybrids.

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Accordingly, Durango teosinte was forced into flower by "short-day treatment" in August 1936, and crossed with maize. The F_1 plants were grown in the greenhouse during the winter of 1936–37. These hybrid plants flowered about the same time as ordinary maize grown in the same greenhouse. F_2 and backcross seed was obtained and planted in the field May 22, 1937, for a preliminary study of the segregation for photoperiod. From the time the first plant shed pollen (August 3rd) until the first killing frost (October 4th) the plants were examined daily and the date of anthesis of each plant was recorded.

The distribution of the dates of anthesis in the backcross to maize is a unimodal curve with the greatest number of plants beginning to shed pollen between August 8th and August 12th. The backcross to teosinte gives

TABLE 1

Date of anthesis and frequency of F_2 plants, 1938.

F_2 PLANT NUMBER 1937	DATE OF ANTHESIS IN 1937	AUGUST				SEPTEMBER						OCTOBER	
		13–17	18–22	23–27	28–1	2–6	7–11	12–16	17–21	22–26	27–1	2–6	7–
3	10/ 2												53
4	9/30												69
9	8/13				1	5	4	0	1	2	1	2	2
8	8/12		1	3	8	14	12	4	6	5	1	4	4
6	8/ 5	1	2	4	4	1	1						
10	8/ 4	1	2	8	3	4	4	2	0	1	1	0	1
2	8/ 3		14	9	7	9	7	1	2	2	1	1	1

a bimodal curve with one mode in August and the other in September. The F_2 also gives a bimodal curve, but the first mode is of much greater magnitude than the second. These F_2 and backcross distributions would be expected if the late-flowering habit of teosinte were determined by a single recessive genetic factor. This response to short-day in teosinte is hereby given the symbol *sd sd* and its allele in maize is designated by *Sd Sd*.

This unifactorial hypothesis was tested further by selfing some F_2 plants and growing their F_3 progeny under similar field conditions the next year. The two modes in the F_2 distribution can be represented by (1 *Sd Sd*+ 2 *Sd sd*) as the major mode, and (1 *sd sd*) as the minor mode. The chances are 1:2 that any F_2 plant taken from the major mode will be homozygous for the maize character, and the chances are 1:0 that an F_2 plant from the minor mode will be homozygous for the teosinte response to short day.

Table 1 shows that F_2 plants numbers 3 and 4, which were in the minor mode in 1937, gave, on selfing, a total of 122 late-flowering plants. This

indicates that these plants were homozygous for the teosinte response to short day, *sd sd*. F_2 plant number 6 gave a unimodal F_3 population, and therefore must have been homozygous for the maize character *Sd Sd*. Plants 9 and 8, however, gave a bimodal curve similar to the original F_2 population. These two plants must have been heterozygous, *Sd sd*. The F_3 populations from plants 10 and 2 are merely skewed to the left and are difficult to explain on the basis of a single genetic factor.

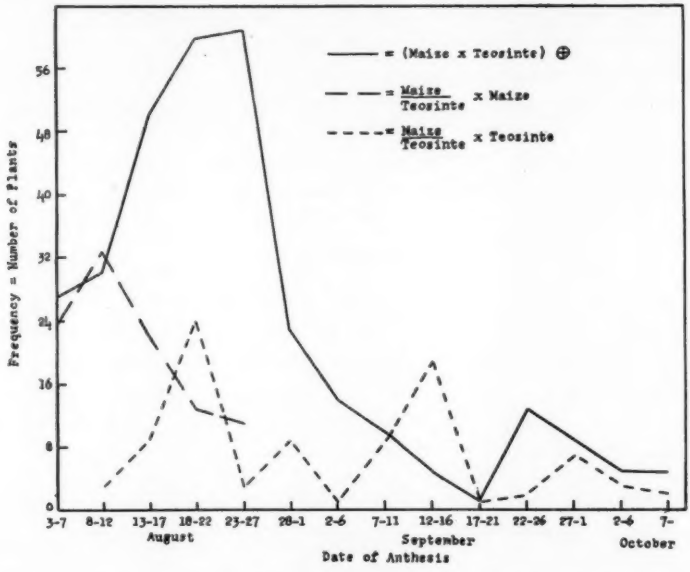

FIGURE 1.—The frequency distribution of the dates of anthesis of F_2 and backcross plants of a maize-teosinte hybrid (1937). The calendar from August 3rd to October 6th has been divided into 5-day intervals instead of daily intervals to make a smoother curve. This grouping has not destroyed any of the major modes.

In this discussion I have referred to the response of maize to length of day as the "maize character." Can it be said that maize is either a long-day or a short-day plant? Probably the best answer to that question is that maize is less sensitive to length of day than any truly long-day or short-day plant. We know that maize will flower in either the long days of the northern latitudes or the short days of the southern latitudes. We also know, however, that a strain of maize should not be moved more than 100 miles north or south from the region in which it is commonly grown. If moved south, it comes into reproduction before it has made suitable vegetative growth. If moved north, it produces good vegetative growth but

does not set seed early enough to escape killing frosts. In other words, for each latitude, strains of maize are selected which have the proper balance between vegetative growth and time of flowering for maximum production in that particular seasonal day length. These strains are referred to as having "medium" maturity. Other strains unadapted to a particular region are said to be either "early" or "late" strains. The early strains would

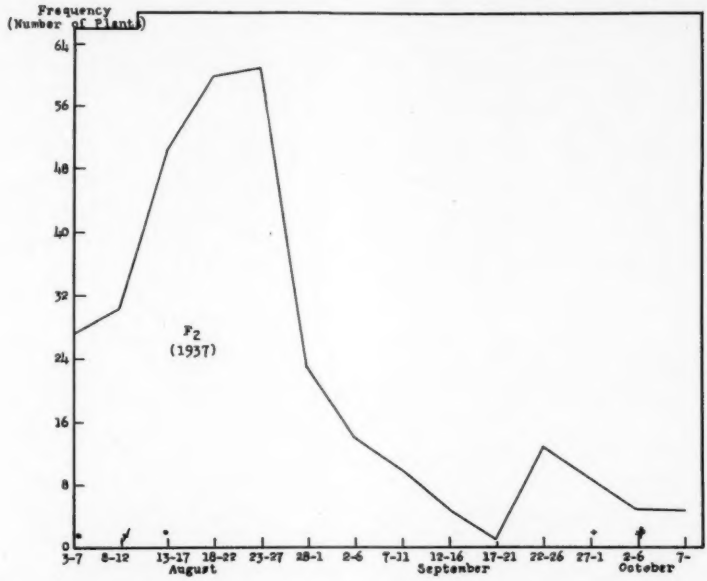

FIGURE 2.—The graph shows the frequency distribution of the dates of anthesis of 313 F_2 plants of a maize-teosinte hybrid in 1937. Seven of these F_2 plants (whose dates of anthesis are indicated) were selfed and their F_3 progeny grown in 1938. Table 1 shows the frequency distribution of the dates of anthesis of the F_3 plants. A killing frost on October 7th marked the completion of the experiment.

be more productive if moved farther north to longer days; whereas the late strains would be more productive if moved farther south to shorter day conditions. The above statements are made from observation of common cultural practices. In order to obtain more concrete evidence, however, an experiment was designed to study the effect of length of day on early, medium, and late strains of maize at Ithaca.

On May 19, 1938, a greenhouse bench 19 feet long by 5.5 feet wide was filled with soil and planted to ten seeds each of early, medium, and late-maturing maize, and two kinds of teosinte: Huixta (from Guatemala) and Durango (from Mexico). On June 9th when the seedling plants were

three to seven inches high, the bench was divided into five sections with black cloth. Each section included ten plants: two plants each of the five types listed above. (See figure 3.)

	6 hours		8 hours		10 hours		12 hours		14 hours	
Early maize	E	E	E	E	E	E	E	E	E	E
Medium maize	M	M	M	M	M	M	M	M	M	M
Late maize	L	L	L	L	L	L	L	L	L	L
Huixta teosinte	H	H	H	H	H	H	H	H	H	H
Durango teosinte	D	D	D	D	D	D	D	D	D	D

FIGURE 3.—Experimental design to test the effect of length of daily light period on time of flowering of maize and teosinte. The table represents a greenhouse bench 19 feet × 5½ feet. Letters indicate the types and positions of the plants tested.

The black cloth was used to shade the sections a part of each day. The plants in section 1 were given only six hours of light daily. Sections 2, 3, 4, and 5 received 8, 10, 12, and 14 hours of light, respectively. This experiment was conducted throughout the growing season, from seedling stage to anthesis. The results of the test are given in table 2.

TABLE 2

Effect of length of day on time of flowering of maize and teosinte. The figures in the table are the days from seedling stage (three to seven inches) to first day of anthesis. The total number of days from date of planting to anthesis can be obtained by adding 21 days to each figure.

	6 HOURS	8 HOURS	10 HOURS	12 HOURS	14 HOURS
Early maize	37	38	36	36	39
Medium maize	43	43	43	43	50
Late maize	62	61	60	58	70
Huixta teosinte	101	101	112	120	—
Durango teosinte	42	42	41	40	74

By following each line of figures across from left to right we can see that there is no appreciable difference in time of flowering of plants exposed to 6, 8, 10, or 12 hours of light daily, except possibly in Huixta teosinte. But the increase in day length to 14 hours, delays the time of flowering of medium and late maize for periods of seven and ten days, respectively. Under the conditions of this experiment, then, maize shows some response to length of day. (Since only a small number of plants were tested, the results are interpreted as indicating direction of reaction, rather than degree of response.) Perhaps an increase in day length to 16 or 18 hours would have further delayed the time of flowering.

It will be noted from table 2 that the date of anthesis of Durango teosinte was delayed 34 days by increasing the day length from 12 to 14 hours. The Huixta teosinte (from Guatemala) flowered earliest in the shorter day lengths, and not at all in the 14-hour chamber. Guatemalan teosinte

would be expected to require a shorter day for reproduction than Mexican teosinte because of the shorter days of its natural habitat.

In this experiment Durango teosinte required 42 days of short-day treatment for reproduction, whereas Guatemalan teosinte required a minimum of 101 days. In a previous experiment (table 3) Durango teosinte flowered in about 35 days after short-day treatment was begun, and Guatemalan teosinte flowered in about 47 days.

TABLE 3

Effect of short-day treatment on teosinte strains.

TEOSINTE	DATE OF PLANTING	NUMBER OF PLANTS	SHORT-DAY TREATMENT BEGUN	SILKS	POLLEN	DAYS TO SILKS
Mexican—						
Durango	May 30	4	July 13	Aug. 16	Aug. 19	35
Nobogame	May 30	8	July 13	Aug. 12	Aug. 16	31
Novocayan	May 30	3	July 13	Aug. 15	Aug. 19	34
Guatemalan—						
Huixta	May 30	13	July 13	Aug. 28	Sept. 1	47

The essential difference between these two experiments is that in the first case, short-day treatment was begun when the plants were in the seedling stage; whereas in the second case, the plants were about six weeks old before being submitted to shorter day conditions. The necessity for longer treatment of seedlings might be explained by assuming that a teosinte plant must attain a certain stage of development before short-day treatment is effective in stimulating reproduction.

One problem which complicates the study of photo-periodism in maize-teosinte hybrids is the separation of earliness and lateness factors from response to length of day. If an F_2 plant from a maize-teosinte hybrid flowers in the latter part of the season, does it flower late because of lateness factors or because it needs the shorter days of the late summer to stimulate reproduction?

This question cannot be answered satisfactorily from any of the data obtained by the writer. One method of approach to the question, however, was to study the relative dates of anthesis of maize, teosinte, and their F_1 hybrid under long-day conditions. The results of this experiment are shown in figure 4.

The F_1 hybrid flowered somewhat later than the parental maize, and much earlier than the parental teosinte. Is this F_1 distribution due to partial dominance of the maize character, or to lateness factors from teosinte? If we could be sure that teosinte contributed lateness factors to the hybrid, then the delayed F_1 curve would be attributed to those factors.

On the other hand, if we could be sure that the flowering date of teosinte, irrespective of response to length of day, were similar to that of the parental maize, which is of medium maturity, then it could be said that the weak response to length of day in maize is not completely dominant over the strong response to length of day in teosinte.

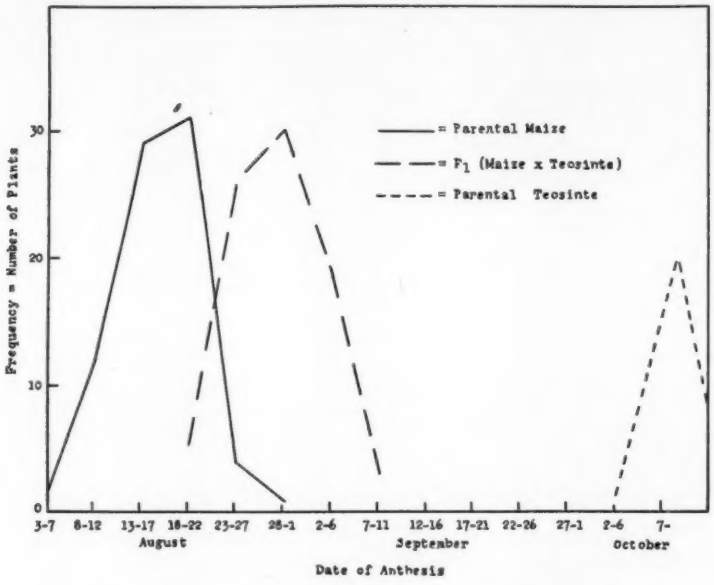

FIGURE 4.—A comparison of the frequency distributions of the dates of anthesis of maize, teosinte, and their F_1 hybrid.

Further information could be obtained by growing maize, teosinte, and their F_1 hybrid in the greenhouse in winter under short-day conditions. This test was not satisfactorily completed because the light in winter was so weak that it had to be supplemented with mazda lights. Artificial illumination was given the plants from 4 P.M. to 10 P.M. each day. The dates of anthesis were recorded and are given in table 4.

The F_1 hybrid flowered earlier than either the maize parent or the teo-

TABLE 4

	DATE OF PLANTING	DAYS TO POLLEN
Parental maize	Dec. 20, 1936	122 (mean of 7 plants)
Parental teosinte	Dec. 20, 1936	176 (mean of 2 plants)
F_1 hybrid	Dec. 20, 1936	100 (mean of 8 plants)

sinte parent. The maize was highly inbred and relatively weak. Perhaps the more rapid development of the F_1 hybrid was due to its advantage in vigor.

LINKAGE RELATIONS OF *sd*

Since the response to short day in teosinte is controlled by a simple Mendelian factor, *sd sd*, and since crossing over between the teosinte chromosomes and their maize homologues is normal (EMERSON and BEADLE 1932), the linkage relations of *sd sd* with qualitative maize characters should show the locus of the *sd sd* gene.

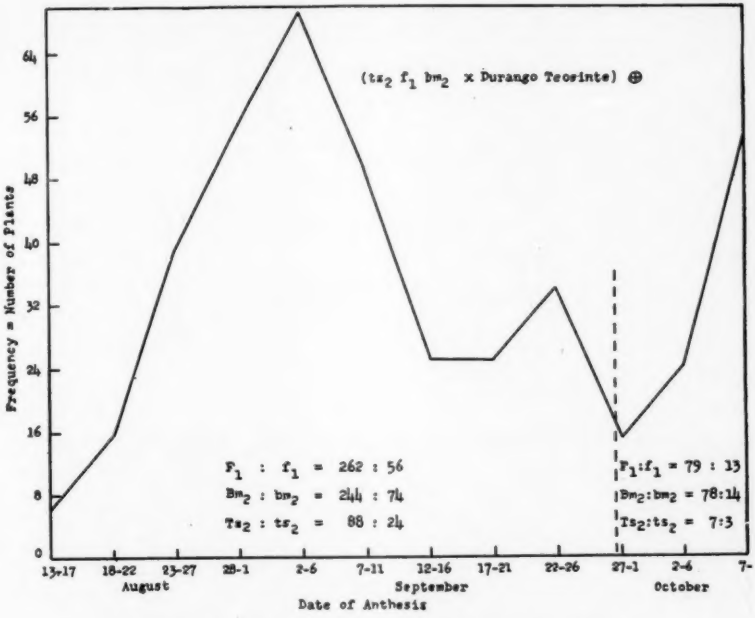

FIGURE 5.—Test for linkage of *sd sd* with genes in chromosome 1.

For this study, teosinte was crossed with tester stocks of maize for chromosomes 1–6, inclusive, in the summer of 1937. The F_1 plants were grown in the greenhouse during the winter of 1937–38 and F_2 seed was obtained. The seed did not mature until late spring, however, and could not be planted until June 3. That was about 18 days later than the most desirable time of planting. This delay was accentuated by two weeks of drouth, and the plants did not appear above ground until the latter part of June. This late start of the seedlings caused the first mode of the dates of anthesis to partially overlap the second mode. The date of the second

mode apparently was not substantially changed because, as mentioned previously, the 13-hour day begins about September first, and 30 days of short-day treatment are sufficient to bring teosinte (or in this case, an F_2 segregate) into reproduction. Actually, then, the second or minor mode was held constant, while the first or major mode was advanced toward it about one month. (See figures 5 to 10.)

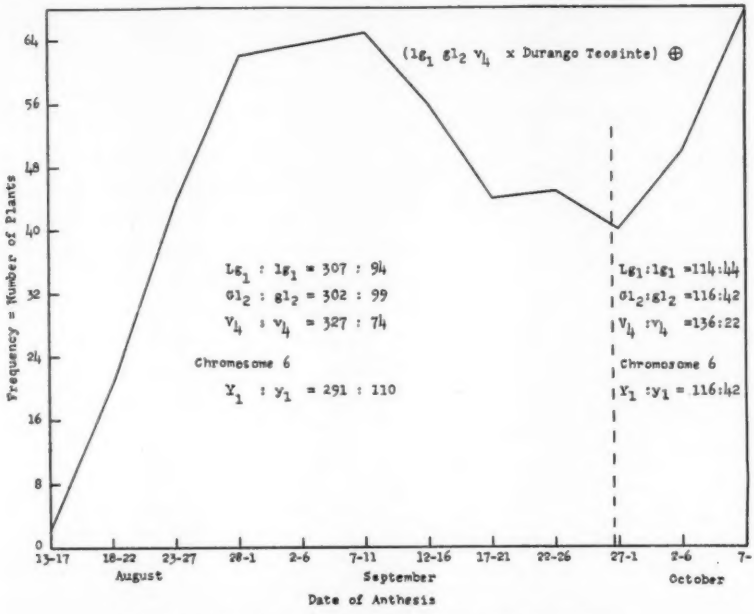

FIGURE 6.—Test for linkage of *sd sd* with genes in chromosome 2.

The method of testing for linkage between the gene *sd sd* (short day) and qualitative maize genes whose loci are known, was to study the segregation of the known genes in the major mode and the minor mode of the F_2 distribution. Assuming that the minor mode consists of homozygous *sd sd* plants, while the major mode is made up of both *Sd Sd* and *Sd sd* plants, independent inheritance of *sd sd* with respect to a known maize gene, *A-a*, would be shown by a 3:1 ratio of *A-a* in each mode.

The data in figures 5–10 show that *sd sd* is not linked with any of the marker genes in chromosomes 1, 2, 3, 4, 5, and 6 used in this study.

DISCUSSION

This study of photoperiodism in Zea-Euchlaena hybrids is genetical, not physiological.

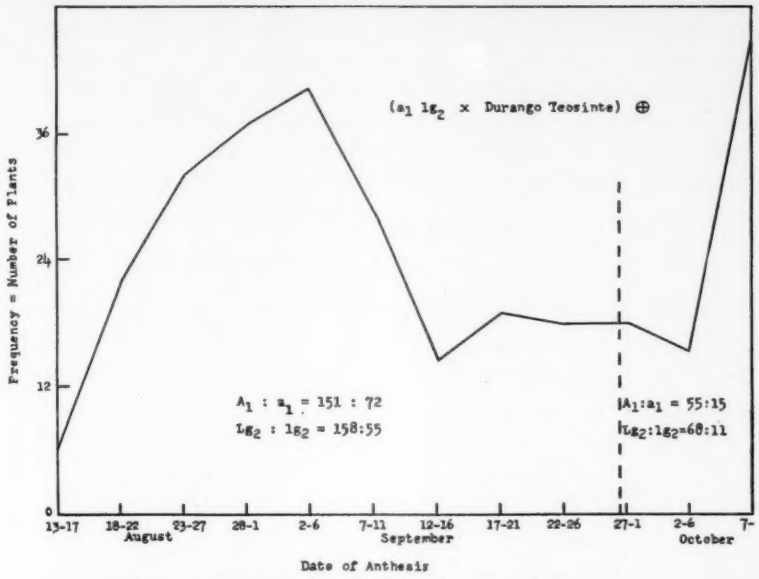

FIGURE 7.—Test for linkage of *sd sd* with genes in chromosome 3.

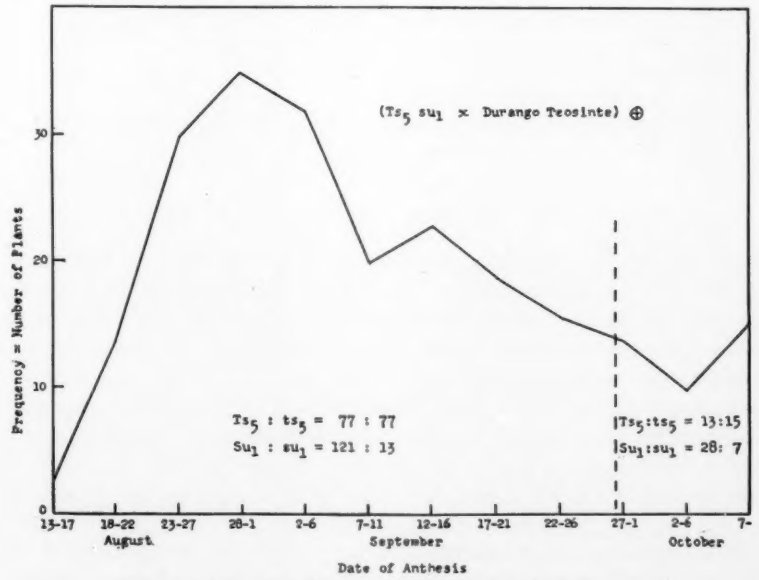

FIGURE 8.—Test for linkage of *sd sd* with genes in chromosome 4.

Perhaps the most convincing evidence of the simplicity of the inheritance of the response to short day is shown by the data in figures 1 and 2. The distribution of the dates of anthesis in F_2 and reciprocal backcross populations follows a unifactorial pattern. This evidence of Mendelian inheritance is enhanced by the breeding behavior of F_2 plants taken at different points in the bimodal distribution. F_2 plants from the second mode gave, on selfing, F_3 populations whose dates of anthesis formed a

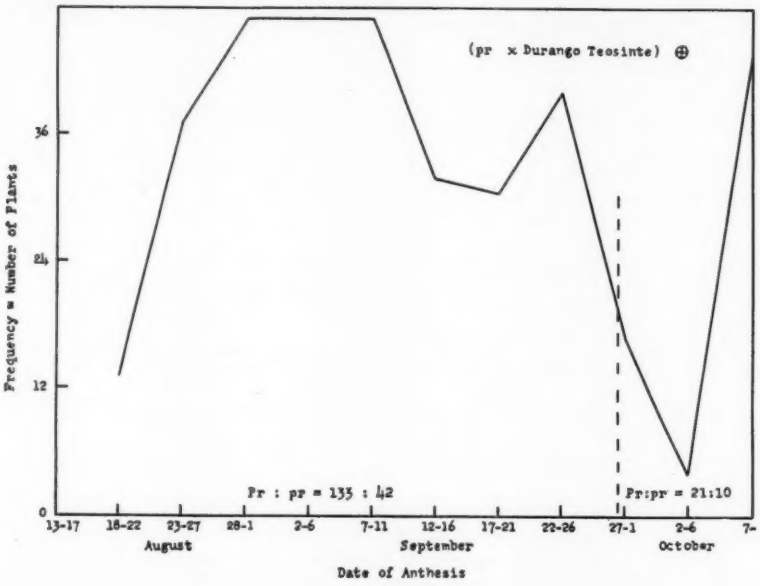

FIGURE 9.—Test for linkage of *sd sd* with genes in chromosome 5.

similar late mode; while F_2 plants from the first mode gave, on selfing, F_3 populations whose dates of anthesis simulated the original bimodal F_2 distribution. The F_3 population from one F_2 plant in the first mode, gave a unimodal curve similar to the first mode, indicating that the selfed F_2 plant was homozygous *Sd Sd*.

If, in maize, the normal allele, *Sd Sd*, of the recessive teosinte response to short day, *sd sd*, mutated to the teosinte form, late-maturing segregates would occur in the ordinary field of corn. Evidence that this or a similar mutation does occur has been presented by three authors: (1) BRUNSON (1935) who reported a plant which gave indefinite vegetative growth under field conditions, with no evidence of either tassel or ear shoot. It could be brought into flowering under short-day conditions. (2) BRYAN (Maize

Cooperation News Letter, March 1938) who described a tall, late-maturing type of plant, which when crossed with medium-maturing types, gave normal F_1 plants; and (3) EMERSON (unpublished), who has informed the writer of a similar late-maturing type, which under Ithaca conditions, will segregate as a simple recessive.

BRUNSON's late-flowering type, *cz*, has been lost due to inviability of old seed stocks. F_2 seed of the types reported by BRYAN and by EMERSON was

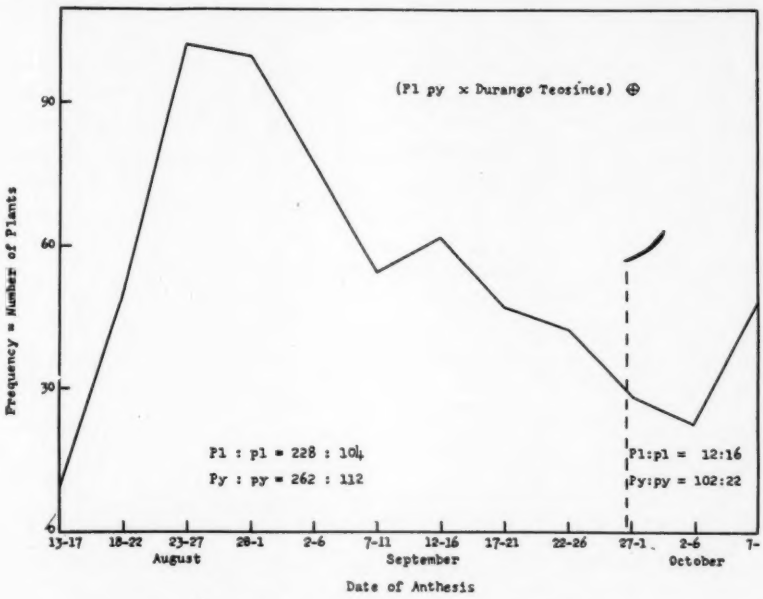

FIGURE 10.—Test for linkage of *sd sd* with genes in chromosome 6.

grown in Ithaca in 1938 and late-flowering plants segregated in a Mendelian manner. One of the segregates from Bryan's stock was crossed with Durango teosinte to test for allelism with the teosinte response to short day. The test will be made in 1939.

Summarizing this section we may say that the response to short day in teosinte is controlled by a simple Mendelian factor, and that mutations to short-day plants occur in ordinary maize.

INHERITANCE OF SPIKELET DIFFERENCES

Paired versus single female spikelets

COLLINS and KEMPTON (1920) reported that of the 33 characters studied in an F_2 population from a cross between Tom Thumb pop corn and Flor-

ida teosinte, the nearest approach to Mendelian inheritance was shown by the arrangement of the spikelets in the pistillate inflorescence. In maize the female spikelets are borne in pairs; in teosinte the female spikelets are borne singly. Dominance of the maize character was complete in the first generation. In the second generation the segregation was not complete; many plants had both single and paired female spikelets, but the number of individuals in which paired spikelets predominated was approximately three times (34 paired to 12 single) the number in which there were more single female spikelets.

Data obtained by the writer from crosses between maize and Durango teosinte agree with the findings of COLLINS and KEMPTON that paired versus single spikelets is a unifactorial difference, but disagree in showing that dominance of paired spikelets is not complete in the F_1 hybrid. Most of the F_1 ears have paired spikelets, but some of them have single spikelets.

TABLE 5

Segregation of paired and single spikelets in F_2 and reciprocal backcross populations from maize-teosinte hybrids.

	F_2		$F_1 \times$ MAIZE		$F_1 \times$ TEOSINTE	
	OBS.	CALC. (3:1)	OBS.	CALC. (1:0)	OBS.	CALC. (1:1)
Paired spikelets	921	904.5	142	142	23	21
Single spikelets	285	301.5	0	0	19	21

In the F_2 population three types of plants are found: those with all single spikelets, those with all paired spikelets, and those with both paired and single spikelets. The ratio of these three types is approximately 1:1:2. In the backcross to maize, all the ears have paired spikelets. In the backcross to teosinte, approximately one-half of the plants have all single spikelets, and one-half of them have both paired and single spikelets.

If, then, plants with both paired and single spikelets are grouped in the class with those plants having all paired spikelets, simple ratios are obtained (table 5).

The data in the above table confirm the hypothesis of unifactorial inheritance of paired versus single spikelets as described by COLLINS and KEMPTON.

The recessive single spikelet condition of teosinte is given the symbol, *pd pd*, and its allele in maize is *Pd Pd*.

The data in table 6 show that an F_2 plant with single spikelets will breed true for single spikelets in the F_3 generation; whereas an F_2 plant with paired spikelets may either breed true for paired spikelets in the F_3 or segregate for single spikelets.

TABLE 6

Segregation of paired and single spikelets in F_2 populations from maize-teosinte hybrids.

F_2 PLANT NUMBER	DESCRIPTION OF F_2 PLANT	NO. OF F_3 SEGREGATES		PROBABLE GENOTYPE OF F_2 PLANT
		PAIRED	SINGLE	
1	Paired Spikelets	38	0	*Pd Pd*
8	Single Spikelets	0	55	*pd pd*
9	Single Spikelets	0	15	*pd pd*
33	Single Spikelets	0	81	*pd pd*
2	Paired Spikelets	42	6	*Pd pd*
6	Paired Spikelets	10	1	*Pd pd*
10	Paired Spikelets	10	10	*Pd pd*
12	Paired Spikelets	24	7	*Pd pd*

Single spikelets, *pd pd*, shows independent inheritance with the marker genes for chromosomes 1, 2, 3, 4, 5, and 6 used in this test. *Lg2* and *pd* showed only 31 percent recombination with a P value of 0.015 and should be tested further (table 7).

Since the difference between the paired and single spikelet condition is controlled by a single genetic factor, a mutation from paired to single spikelets might be expected to occur in ordinary maize. The chances that this mutation would be noticed in the resulting plants are not very great, however, especially in a culture with high row numbers. The change would

TABLE 7

Linkage relations of single spikelets, pd pd, with known qualitative maize genes.

CHROM.	GENES	LINKAGE PHASE	NUMBER OF INDIVIDUALS					PERCENT RECOMB.
			XY	*Xy*	*xY*	*xy*	TOTAL	
1	*F1 Pd*	R	144	39	37	5	225	40
	Bm2 Pd	R	140	34	41	10	225	50
2	*Lg1 Pd*	R	160	38	50	9	257	46
	Gl1 Pd	R	154	34	58	11	257	48
	B Pd	C	40	28	36	11	115	60
	V4 Pd	R	167	38	43	9	257	49
3	*A1 Pd*	R	94	19	42	5	160	43
	Lg2 Pd	R	100	22	36	2	160	31
4	*Ts5 Pd*	C	50	15	18	10	93	41
	Su1 Pd	R	46	26	7	4	83	50
5	*Pr Pd*	R	54	20	20	7	101	49
6	*Y1 Pd*	C	155	35	55	12	257	49
	Pl Pd	C	73	25	34	13	145	48
	Py Pd	R	166	87	37	10	300	41

simply reduce the row number by one-half, and a 16-row type would show a few 8-row ears which would probably be ignored.

A mutation to single spikelets in an inbred line of maize was discovered by the writer when he crossed maize with teosinte. The F_1 ears had single spikelets instead of the usual paired spikelets. The F_2 ears, likewise, were homozygous for single spikelets. The inbred line of maize used in this cross has a fasciated type of ear, and its single spikelets would not have been noticed except for the cross with teosinte.

Summarizing this section, it has been shown that the paired spikelet condition of maize is dominant over the single spikelet condition of teosinte, and is a simple Mendelian character; and that mutation from paired to single spikelets occurs in maize.

INHERITANCE OF TASSEL AND EAR DIFFERENCES

Two-ranked versus many-ranked central branch of the tassel

The two-ranked central branch of the teosinte tassel[2] as contrasted with the many-ranked central branch (or central spike) of the maize tassel presents one of the characteristic differences between maize and teosinte. How is this character inherited in hybrids between the two genera?

The F_1 plant has a many-ranked central branch in its tassel, similar to

TABLE 8

The segregation of the two-ranked teosinte type of central branch in F_2 populations from maize-teosinte hybrids.

F_2 PLANT NUMBER	DESCRIPTION OF F_2 PLANT	NUMBER OF F_3 SEGREGATES		PROBABLE GENOTYPE OF F_2 PLANT
		MANY-RANKED	TWO-RANKED	
9	Two-ranked	0	16	*tr tr*
1	Many-ranked	58	0	*Tr Tr*
8	Many-ranked	59	0	*Tr Tr*
2	Many-ranked	26	13	*Tr tr*
6	Many-ranked	5	6	*Tr tr*
10	Many-ranked	15	3	*Tr tr*
12	Many-ranked	32	18	*Tr tr*

the maize parent. In the F_2 population, the two-ranked teosinte type of central branch segregated sharply as a simple Mendelian character (figure 11). The F_2 population consisted of 890 plants with many-ranked and 316 plants with two-ranked central spikes.

The recessive two-ranked central branch of the tassel is given the symbol, *tr tr*, and its dominant allele, the many-ranked central branch of the maize tassel, is designated by *Tr Tr*.

[2] Contrary to general belief, the central branch of the teosinte tassel is a continuation of the axis of the panicle or rachis, and not a branch from the rachis.

The data in table 8 show that an F_2 plant with a two-ranked central branch will breed true for the two-ranked condition in the F_3 (according to the one F_2 plant tested); whereas an F_2 plant with a many-ranked central branch may either breed true for the many-ranked condition, or segregate for the two-ranked condition.

This study of the teosinte type of tassel as contrasted with the maize

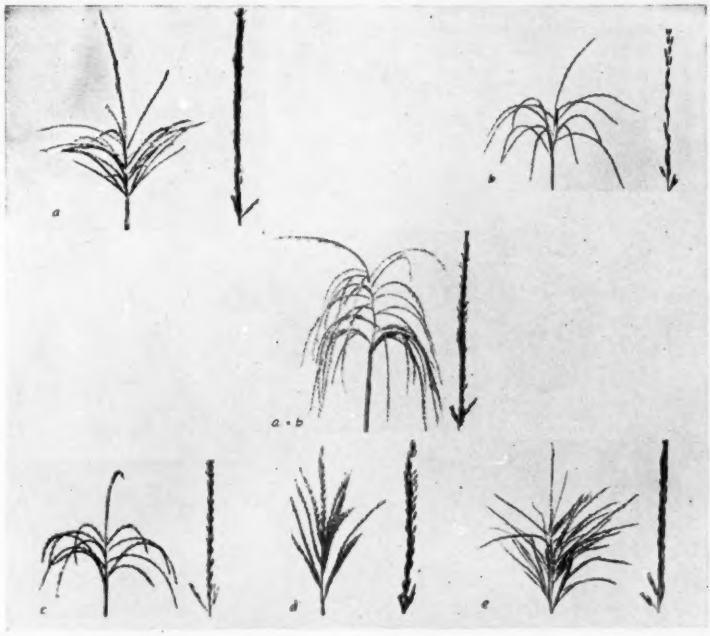

FIGURE 11.—The inheritance of many-ranked (*Tr Tr*) vs. two-ranked (*tr tr*) central branch of the tassel in maize-teosinte hybrids. Inset: enlargement of central branch.
a—parental maize (*Tr Tr*).
b—parental teosinte (*tr tr*).
a×b—F_1 hybrid (*Tr tr*).
c, d, e—Teosinte-like segregates (*tr tr*) in F_2.

type includes only the difference between a two-ranked central branch and a many-ranked central branch. It does not pretend to be a complete study of tassel type. Other factors to be considered in a more detailed study would be total number of spikelets; size of spikelets; number, length, and positions of lateral branches; articulation; and others. The wide range of tassel types in maize is a continuous series which suggests multiple factor inheritance.

The distinction between a two-ranked and a many-ranked central

branch, on the other hand, is discontinuous, and the two types can be readily classified in segregating populations.

Two-ranked versus many-ranked ear type

As will be seen from this study, the two-ranked central branch of the teosinte tassel and the two-ranked ear of the teosinte plant are directly related and are both controlled by the same recessive gene, *tr tr*. Moreover, the many-ranked central branch (or central spike) of the maize tassel and the many-ranked ear of the maize plant are directly related and are both controlled by the same dominant gene, *Tr Tr*.

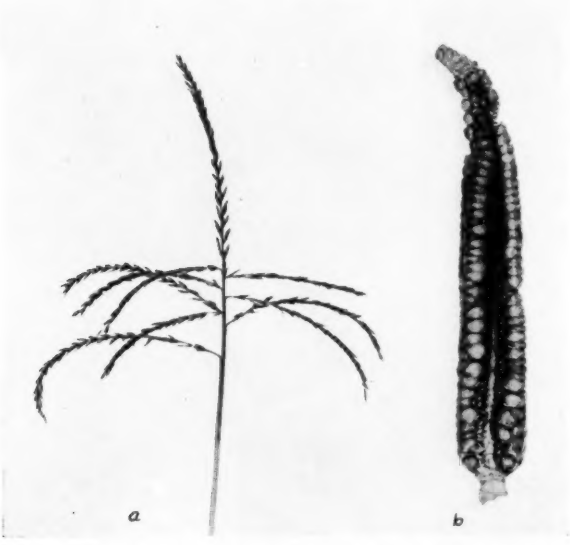

FIGURE 12.—Mutant form of maize with four-rowed (two-ranked) ear and two-ranked central branch of the tassel.

In maize-teosinte hybrids most of the F_1 ears are two-ranked, but a few of them are many-ranked. These two-ranked ears occur laterally on the side branches in the axils of the leaves, while the few many-ranked ears occur terminally on the side branches. (Most of the side branches are terminated by staminate inflorescences, not pistillate inflorescences.) In spite of the predominance of two-ranked ears in the F_1 hybrid, the many-ranked condition of maize is considered dominant over the two-ranked condition of teosinte, because (1) the central branch of the F_1 tassel is many-ranked; (2) in segregating populations, two-ranked ear is associated with two-ranked central branch and many-ranked ear with many-ranked

central branch; and (3) four-rowed (two-ranked) mutant form of maize (see figure 12) has a two-ranked central branch in its tassel: when this mutant form is crossed with ordinary maize (TAVCAR 1935), the F_1 plant has a many-ranked ear and a many-ranked central branch. In the F_2 population the mutant form segregates as a simple Mendelian character. Furthermore, this two-ranked mutant form of maize is allelic with the two-ranked condition of teosinte, as shown by test crosses with teosinte.

TABLE 9

Linkage relations of two-ranked, tr tr, condition with known qualitative maize genes.

CHROM.	GENES	LINKAGE PHASE	NUMBER OF INDIVIDUALS					PERCENT RECOMB.
			XY	*Xy*	*xY*	*xy*	TOTAL	
1	*Fi Tr*	R	140	44	32	9	225	48
	Bm2 Tr	R	127	46	45	7	225	38
2	*Lg1 Tr*	R	144	53	49	11	257	43
	Gl1 Tr	R	138	50	55	14	257	45
	B Tr	C	57	11	38	9	115	47
	V4 Tr	R	156	53	37	11	257	48
3	*A1 Tr*	R	87	26	40	7	160	42
	Lg2 Tr	R	93	29	32	6	160	43
4	*Ts5 Tr*	C	38	4	41	10	93	38
	Su1 Tr	R	61	11	8	3	83	60
5	*Pr Tr*	R	44	30	19	8	101	43
6	*Y1 Tr*	C	145	45	48	19	257	47
	Pl Tr	C	61	37	31	16	145	52
	Py Tr	R	183	80	34	3	300	28

In classifying ear type in an F_2 population from a maize-teosinte hybrid, then, the type of central branch in the tassel serves just as well as the ears themselves. A plant homozygous for the gene, *tr tr*, has a two-ranked ear and a two-ranked central branch. A plant homozygous *Tr Tr* or heterozygous *Tr tr* has a many-ranked ear and a many-ranked central branch.

The F_2 Mendelian ratio and the F_3 ratios in table 8 apply not only to the inheritance of tassel type (two-ranked or many-ranked), but also to the inheritance of ear type.

Two-ranked, *tr tr*, shows independent inheritance with the marker genes used from chromosomes 1, 2, 3, 4, and 5; and possible linkage with pigmy, *py py*, in chromosome 6. Due to the fasciation of the pigmy tassel, this linkage is subject to further test with other genes in that region of chromosome 6.

These data show that *tr tr* and *pd pd* are on the same chromosome, and are 20 crossover units apart.

The number of the maize chromosome which carries the genes *Pd Pd* and *Tr Tr* is not known. The data in table 9 indicate that *Tr Tr* is linked with the gene, *py py*, on chromosome 6. The writer has little confidence in that linkage, however, because of the fasciation of the pigmy tassel.

The 20 percent recombination of *Pd pd* and *Tr tr* makes possible the production of ears with an odd number of kernel rows, that is, F_2 plants with the genetic constitution *pd pd Tr Tr* or *pd pd Tr tr* (single female spikelets, many-ranked) may have any number of rows from 2 to 13, inclusive. Ears with an odd number of rows have been found.

TABLE 10

Linkage relations of two-ranked, tr tr, with single spikelets, pd pd.

GENES	LINKAGE PHASE	NUMBER OF INDIVIDUALS					PERCENT RECOMB.
		XY	*Xy*	*xY*	*xy*	TOTAL	
Tr Pd	C	795	95	126	190	1206	20

In summary of this section, it may be said that the two-ranked ear and the two-ranked central branch of the teosinte tassel are both controlled by the same recessive gene, *tr tr;* and that in maize, the dominant gene, *Tr Tr* (many-ranked) mutates to the recessive form, giving a plant with a two-ranked ear and a two-ranked central branch.

GENERAL DISCUSSION

One of the theories of the origin of maize is that it arose from teosinte by a relatively few large-scale mutations. This theory, however, is not widely accepted. One of the most serious objections raised against it is that the characters which differentiate the two genera are numerous and show multiple factor inheritance, which suggests that maize and teosinte differ by hundreds if not thousands of genes.

Actually, however, when all the characters which show wide variation in both maize and teosinte are omitted, and only the characteristic differences are considered, the list of differences is reduced to about five as follows:

Weak versus strong response to length of day
Paired versus single female spikelets
Many-ranked versus two-ranked ear (and central branch of the tassel)
Membranous versus horny glume
Naked versus covered seed.

Width of leaves, tillering, sturdiness of the plant, and similar characters,

cannot be considered characteristic differences because each shows extreme variation in both maize and teosinte.

As shown by the experimental results outlined in this paper, at least three of the characteristic differences are inherited as simple Mendelian characters. This unifactorial inheritance tends to obviate the speculative suggestion that maize and teosinte differ by hundreds if not thousands of genes, and makes the theory of the origin of maize from teosinte by mutation a plausible one.

Perhaps if a number of inbred lines of teosinte were maintained over a period of years, mutations to maize-like characters would occur and could be combined to synthesize maize. This possibility does not seem fantastic in view of the fact that mutations to teosinte-like characters have been found in inbred lines of maize (paired to single female spikelets, many-ranked to two-ranked condition, and weak to strong response to length of day).

SUMMARY

1. The weak response to length of day in maize is dominant over the strong response to length of day in teosinte, and segregates as a simple Mendelian character. Mutation to the teosinte form occurs in maize.

2. The character paired female spikelets of maize is dominant over single female spikelets of teosinte, and shows simple inheritance. Mutation from paired to single spikelets occurs in maize.

3. The many-ranked ear of maize is dominant over the two-ranked ear of teosinte and segregates as a unifactorial difference. Two-ranked ear is associated with two-ranked central branch of the tassel, and many-ranked ear with many-ranked central branch. Mutation to the teosinte form (two-ranked) occurs in maize.

4. The gene, *pd pd* (single spikelets), is linked with the gene, *tr tr* (two-ranked), with 20 percent recombination.

5. The results are interpreted as supporting the theory of the origin of maize from teosinte by a relatively few large-scale mutations.

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ABSTRACTS OF PAPERS PRESENTED AT THE 1939 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

COLUMBUS, OHIO, DECEMBER 28–30, 1939

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Department of Genetics
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ATWOOD, SANFORD S., U. S. Regional Pasture Research Laboratory, State College, Pa.: *Cytogenetics of incompatibility in Trifolium repens.*——Thirteen self-sterile F_1 plants were crossed in all combinations and were backcrossed with their self-sterile parents. The F_1 plants consisted of four intra-sterile, inter-fertile groups of five, four, three, and one plant, respectively, and all were reciprocally fertile with both parents. Using ten flowers in each cross, compatible crosses averaged 44.9 seeds and incompatible 0.26. A second series of diallel crosses were made between $13 F_1$ plants and their parents. One parent was self-sterile and the other highly "pseudo-self-fertile." Both this latter parent and its progeny were practically self-sterile when individual flowers were self-pollinated in either full flower or the bud, but they set selfed seed when the entire heads were rubbed to effect pollination. The factors conditioning this pseudo-self-fertility are independent of those causing cross-incompatibilities, since four intra-sterile, inter-fertile groups of four, four, three, and two plants, respectively were found in this second set of progeny. Compatible crosses averaged 41.9 seeds and incompatible 0.18. The results in both series of crosses are explained best by the diploid (16 bivalents found regularly) personate type of oppositional factors where the parents differ in both alleles. The 12 groups from the two series were compatible in all combinations, indicating at least seven different allelomorphs in the four parents. Other evidence suggesting a large number of allelomorphs is that all matings (over 200) between unrelated plants were compatible. Few or no seeds were set in incompatible crosses because of poor pollen germination and pollen tube growth within a few hours after pollination.

BANTA, S. M., Brown University, Providence, R. I.: *Possible rôle of amixis in the evolution of organisms.*——Experimental findings from parthenogenetic reproduction in Cladocera and other well authenticated facts suggest that amixis, particularly when alternating with amphimixis, may be a potent factor in evolution. In Daphnia, mutation in diploid parthenogenesis is moderately frequent——all of our recognized Cladocera mutations having arisen during parthenogenesis. Most of these mutations are recessives and their effects, almost exclusively physiological, are recognized only in the results of sexual reproduction——inbreeding within the clone involved. The relative frequency of occurrence of these mutations during parthenogenesis together with, in nature, the periodical occurrence of amphimixis suggests that in the amictic-amphimictic cycle abundant genic recombinations occur.

The vast populations normally resulting from amixis should contain proportional numbers carrying any recessive mutations, or any non-deleterious dominant mutations, which might have arisen. If the mutation is dominant and confers an advantage to its possessor the mutant population during parthenogenesis should become disproportionately large. In any case, if the mutation is of potential advantage the amictic-amphimictic cycle affords opportunity for the one-in-a-million recombination which, perhaps alone, may produce an individual superior to its forbears or fitted for occupancy of a changed or new environment. For example, our thermal mutation in *Daphnia longispina* was such a potentially advantageous mutation. The amictic-amphimictic cycle occurs in many organisms, particularly among parasites and animals occupying transitorally favorable environments. It is conceivable that this mechanism has been a large factor in the development of the almost incredibly specialized and elaborate adaptations in life histories of certain parasites.

BARTO, ELIZABETH, Laboratory of Vertebrate Genetics, University of Michigan, Ann Arbor, Mich.: *Absence of linkage between certain characters in the deer-mouse, Peromyscus maniculatus.*——The characters albino versus dilute, albino versus nude, and ivory versus hairless, in the deer-mouse, *Peromyscus maniculatus*, have been tested to determine their linkage relationships. Independent assortment of the genes for each of these pairs of characters was shown in the F_2 generations and in test-crosses, indicating absence of linkage.

BEATTY, ALVIN V. University of Alabama, University, Ala.: *Mitosis in the pollen tube of Eschscholtzia.*——Within fifteen minutes after sowing the pollen of the California poppy on a prepared medium, both the growth of pollen tubes and the division of generative nuclei have begun. All normal mitotic prophase stages, including the double nature of the chromosomes, can be seen. At the end of two hours the pollen tubes range in length up to 2 mm. Most tubes between 0.5 mm and 1.0 mm show a typical mitotic metaphase with a well developed spindle and an equatorial alignment of the chromosomes. Since the chromosomes are small they are situated throughout their lengths in the equatorial plane. The anaphase chromosomes are separate, easily distinguishable, and migrate simultaneously, forming regular mitotic configurations. Immediately following telophase the gametes are formed. They migrate toward the end of the tube, but are rarely closer together than the distance between the telophase nuclei.

BLAIR, ALBERT P., Indiana University, Bloomington, Ind.: *Inter-relations of the toads of eastern North America.*——Inter-relations of the toads of eastern North America have been studied from three angles: (1) extensive field work involving determination of ranges, areas of hybridization, etc., (2) critical

examination of specimens collected in the field, and (3) experimental hybridization. The several so-called species are found in almost every case to be linked by intermediate populations. 3500 toads have been measured and local variation studies made where sufficient specimens are available. 987 species hybrids representing 29 crosses have been raised through metamorphosis.

BLAKESLEE, A. F., AVERY, A. G., and BERGNER, A. D., Carnegie Institution of Washington, Cold Spring Harbor, New York: *Genes associated with prime types in Datura and their possible relation to the hypothesis of position effect.*—— In our collection of over ninety prime types which consists chiefly of races homozygous for segmentally interchanged chromosomes, the large majority show no deviation from normal in appearance as would be expected if position effect were frequent. In a few cases, however, homozygous prime types are distinctly abnormal. It has not yet been possible to determine whether these exceptions are due to genes which do not cross over, to small deficiencies which accompanied the break, or to position effect.

BLANC, RICHARD, and BRAUN, WERNER, Department Zoology, University of California, Berkeley, Calif.: *Phenocopies and X-radiation in Drosophila melanogaster.*——The authors have attempted to establish a relationship between number of phenocopies in *Drosophila melanogaster* and extent of X-radiation. Prepupae of an inbred Oregon-R stock were subjected to X-radiation for periods of $\frac{1}{4}$ to $4\frac{1}{2}$ minutes (at approximately 940 r units per minute), and the images were examined for phenocopic effects. Characters most frequently noted were abnormal abdomen, various bristle defects (particularly absence), curled and curved wings, opaque wings, and trident. Occasional phenotypes included rough eyes, vortex, and a number of wing shape and wing venation effects. A direct proportionality between average number of phenocopies per fly and degree of radiation was observed from 1410 r units to the highest dosage for which significant data were available, i.e., 3290 r units. The decrease in number of normal flies is represented by an S curve. A typical mortality curve was found, with a sharp drop in viability from 2350 to 3290 r units. Certain relationships were established among the thoracic bristles. The anterior and posterior dorsocentrals reacted differently to increase in radiation, while the anterior and posterior scutellars reacted in a similar manner, though not to the same degree. It is suggested that the scutellars may be classified as a single bristle system, and the anterior and posterior dorsocentrals as separate bristle systems.

BRAUN, WERNER, Department of Zoology, University of California, Berkeley: *Increase in time of development after partial and complete starvation of larvae and its effect on the phenotype of several mutants of D. melanogaster*——Partial (peptone-food) and complete starvation of larvae of *D. melanogaster* increases time of development. Developmental rate can be changed differently accord-

ing to the degree and length of starvation and the stage in development at which it is started. If the time of development is thus increased in vg^{no} larvae an increasing destruction of wing area can be observed with increasing time of development (Proc. Nat. Acad. Sci. **25,** 5). Precisely timed tests indicated that no definite sensitive period exists for the effect of partial starvation. The effect is larger the earlier the larvae are transferred to peptone food. After partial starvation the destruction always increases from the tip of the wing towards the base of the wing (scalloping).——No definite sensitive period for complete starvation has been found as yet. *Patterns* of destruction are typically different if starvation is started severally at different times of larval development. Larvae which are completely starved for a 3 day period, which starts before the larvae are 75 hours old, develop into flies 80% of which always exhibit a destruction starting from the posterior margin of the wing (*Bd* type). If larvae are completely starved beginning at a time after 75 hours of larval age, all hatching flies exhibit wing destruction starting from the anterior margin (*Bx* type). An antler-like pattern of destruction results (60% of hatching flies) if larvae were removed from all food at the age of 50 hours. Changes of developmental rates were produced by the same method in some other mutants of *D. melanogaster*. It was observed that after increase in time of development *B*/+ and Lobe eyes become normal, Curly wings straighten out (only in certain stocks) and the amount of extra venation is reduced in plexus stocks.

Brehme, Katherine S., Carnegie Institution, Cold Spring Harbor, N. Y.: *The growth of transplanted Minute and wild type optic disks in Drosophila melanogaster.*——As part of a study of the effect of the host upon growth of imaginal tissue, optic disks of female larvae 76 hours after hatching were transplanted into larvae of the same age and sex (at 25°C). At eclosion of the host, the transplants were dissected out and the facets counted. The stocks used were Florida wild type (puparium formation at 100 hours after hatching) and Minute-w isogenic with Florida (puparium formation at 144 hours). The mean facet number of *Mw* transplants in wild type hosts, pupating 24 hours after the operation, was 294.3 ± 31.8; *Mw* transplants *Mw* hosts, pupating 68 hours after the operation, had a mean of 487.3 ± 43.1 facets (difference = 193.0 ± 51.9). Wild type disks in wild type hosts, pupating 24 hours after transplantation, formed a mean of 489.1 ± 28.8 facets; in *Mw* hosts, pupating in 68 hours, they formed 599.5 ± 23.1 facets (difference = 110.4 ± 36.9). The following conclusions may be drawn: 1) The length of the interval between transplantation and puparium formation of the host, during which the transplant increases in cell number, is a major factor in determining the size attained by the transplanted disk. This is in agreement with data on Bar obtained under different conditions by Bodenstein. 2) Increase in cell number continues in the wild type optic disk after 76 hours (data from+in+and+in *Mw*). 3) Under conditions of the experiment, the wild type disk forms fewer facets in transplant than in situ.

BRINK, R. A., and COOPER, D. C., University of Wisconsin, Madison, Wis.: *The significance of double fertilization in flowering plants.*——The early collapse of fertile ovules in *Medicago sativa* is about five times as frequent following selfing as after cross-pollination. This type of seed failure, which is termed somatoplastic sterility, is shown to be due primarily to excessive meristematic activity of the inner integument, causing starvation of the endosperm and embryo. Continued development of the young seed apparently requires that the endosperm maintain an ascendant physiological position relative to the growing maternal tissue, (nucellus or integument, as the case may be), adjacent to the embryo sac with which it must compete for a limited food supply. Otherwise the partition of nutrients becomes unbalanced in favor of the nucellus or integument, and somatoplastic sterility results. Marked heterosis is shown in the young hybrid endosperm, although it can not be detected in the much more slowly growing embryo. Conversely, inbreeding depresses the rate of nuclear division in the alfalfa endosperm, in many cases below the critical level for ovule survival. Double fertilization, therefore, may be interpreted as a mechanism which, through heterosis, enhances the competitive position of the endosperm in the delicately balanced internal environment of the ovule.

BRYSON, VERNON, Columbia University, New York.: *The modifying effect of Minutes.*——A wide variety of mutant genes have been combined with $M(3)$ and its allele $M(3)Fla$, with the purpose of determining the effect of extreme Minutes on dominance, penetrance and expressivity. Particular interest is attached to experiments involving $B^{i}B^{i}$, Bx^{3}, *co*, *fa*, ap^{4}, *Jag*, *Ser*, *Lyra* and *Xa*, combined individually with these Minutes. Here the modifying action of Minutes was well marked, whereas in numerous other cases no appreciable modifying influence could be detected. It is shown that Minutes often enhance the phenotypic expression of genes with which they are combined and that such modification is consistent with the generally accepted viewpoint that dominance, penetrance and expressivity are aspects of the same problem. In the heterozygote detection of modification involving any of these phenomena is correlated with the expression of one, as contrasted with two doses of the gene, in the non-Minute genotype. Enhancement must obviously be in the direction of the homozygote; e.g., *Jag/MFla* is phenotypically similar to *Jag/Jag*, not to *Jag/+*. Entirely consistent is the manifestation of phenotypic effect by confluens, which in the non-Minute heterozygote is undetectable, but in the Minute appears as though homozygous. Similarly penetrance may be increased. Modification of the homozygote is also in the direction of enhanced expression which may now be recognized as beyond the limits imposed by the normal residual genotype. If the homozygote is lethal but has a phenotypic effect in the heterozygote, then its heterozygous combination with Minute may fail to survive, as in *MFla/Dfd* (Payne). These experiments indicate that Minutes may be regarded as among the most effective of nonspecific modifiers in Drosophila.

COOPER, D. C., and BRINK, R. A., University of Wisconsin, Madison, Wis. *Somatoplastic sterility as a cause of seed failure following interspecific hybridization.*——Fertilization occurs between 50 and 60 hours after pollination in the following matings: *Nicotiana rustica* × *N. glutinosa*, and reciprocal, *N. rustica* × *Petunia violacea* and *N. glutinosa* × *P. violacea*. Seed is not formed, however, due to early collapse of the fertile ovules. Histological changes leading to failure are essentially like those frequently observable in normally cross-pollinated *Medicago sativa* after selfing, as earlier described by the authors under the term somatoplastic sterility. Normal seed formation follows self-pollination in *N. rustica* and *N. glutinosa*. Fertilization occurs 20–24 hours after pollination, and endosperm and embryo develop rapidly. Nucellar cells become flattened, forming a thin and gradually shrinking layer between endosperm and integument. The course of development following interspecific and intergeneric hybridization, on the other hand, is conspicuously different. The endosperm particularly, and possibly the embryo also, grow more slowly. The nucellus quickly becomes meristematic, instead of regressing, and forms two or more layers of cells. As the nucellar cells proliferate, starch appears in them, the endosperm breaks down and development of the ovule eventually ceases. The immediate cause of ovule collapse is attributed to endosperm starvation due to hyperplasia of the nucellus. The latter condition is believed to arise when the genetic constitution of the endosperm is such that it fails to attain and maintain an ascendant physiological position relative to the adjacent maternal tissue which is also stimulated to development following fertilization.

CUMLEY, R. W., and IRWIN, M. R., University of Wisconsin, Madison, Wis.: *Immunological and geographic relationships among pigeon species.*——Using immunological procedures, notably the agglutinin-absorption technic, it was found that the species of pigeons from the Old World and those from the New World, when compared, represented two more or less distinct groups. Briefly, there appear to be antigens which are common to species of both the Old and the New World. Further, within the species of the Old World there are other antigens which set these species apart as a group from any of those in the New World. These components are shared by many , if not by all, of the Old World species. And then there are other antigens which set each species apart from every other species of the Old World. Likewise, the species of the New World have a group of cellular characters which are not found in those of the Old World, and which are present in most, if not in all, of the species of the New World. Also, each species has its own distinctive antigens.

CURTIS, M. R., and DUNNING, W. F.; Institute of Cancer Research, Columbia University, New York, N. Y.: *An independent recurrence of the blue mutation in Rattus norvegicus and observations on a mosaic of blue and its normal allelomorph.*——A recurrence of the blue mutation described by ROBERTS in 1929 is reported in a strain of pedigreed agouti black hooded rats. The foundation

stock of this strain was three rats obtained from Copenhagen in 1920. The known genetic constitution of the strain is *CC PP AA hh*. The first observed blue mutants appeared in the 15th brother × sister generation of Line 2331. Prior to this time Feb. 23, 1934 intense hooded coat color had been recorded for each of the observed 6059 rats of this strain of which 187 were direct ancestors and their full sibs and 504 were half-sibs of the direct ancestors of the mutants. Since the appearance of the mutation the collateral families have produced 3565 pure line intense colored progeny and thousands of hybrids none of which have shown the mutant character. The identity of this blue character with the one observed by ROBERTS has been established by CASTLE in crosses of the two stocks. A hooded female mosaic for blue and intense color has recently been observed in an F_2 population of 286 rats from a cross of intense hooded (*Dh*) by blue selfed (*dH*). Her pattern is 26 percent colored with a black area on the right side of the face and left side of the cape equal to about 10 percent while the remainder of the colored area is blue. She has had to date 16 blue progeny by a blue selfed male indicating that the mosaicism is somatic.

DAWSON, W. M., and KATZ, REUBEN, U.S.D.A., Bureau of Animal Industry, Washington, D. C.: *Preliminary report on variation in ability of dogs to master a multiple-choice situation.*——As a basis for later genetic studies an attempt is being made to find types of behavior that will provide a clear-cut discrimination in the intelligence of dogs. A modification of the "Hamilton Multiple-Choice Apparatus" is used in one of the tests. The dog is faced with the problem of finding which one of four doors is unlocked. In these tests many dogs developed a good system of seeking the right door which eliminated the repetitions that would otherwise have been expected. Pulis, Chows, Border Collies and F_1's of Puli × German Shepherd, Puli × Chow, and Puli × Border Collie, totaling 44 dogs, were tested. When dogs were scored on avoidance of repetition, a wide distribution of scores was obtained. On the basis of total doors tried in approximately 140 trials by each dog the percent of doors not repeated varied from 39 to 80. The mean for the 44 dogs was 56.8 ± 0.9. Average scores for the three largest groups were: Pulis, 54.3 ± 2.3; F_1 (Puli × German Shepherd) 62.5 ± 1.3, and F_1 (Puli × Chow) 55.2 ± 1.5. While the means of the first two groups appear to differ significantly the magnitude of the reliability coefficient of the test ($r = .82$) and the small number of dogs in each group make it necessary to do further work before drawing final conclusions. One litter (Puli × F_1 (Puli × German Shepherd)) in which the F_1 dam was selected for her very high score on this test (80), has an average score of 63.9 ± 2.0 indicating that the selection may have had some effect.

DEMEREC, M., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *A comparison between the X-ray induced and the spontaneous Notches.*——Salivary gland chromosome study of an unselected series of 27 X-ray induced Notches shows that 3 had the full complement of bands, in 7 cases one

band was missing, in 4 cases 2 to 5 bands were missing and in 13 cases more than 6 bands were missing. Similar study of a series of 10 spontaneous Notches shows 1 case in the first group, and 3 cases in each of the other three groups. The spontaneous series consisted of Notches kept in stock at various laboratories and obtained from them for this study. Since long Notch deficiencies have poor viability it is probable that in this series natural selection may have eliminated some of the long deficiencies. Such a possibility finds support in the genetic analysis by LI and BRIDGES of spontaneous Notches collected at random; it was found that 4 out of 25 involved the white locus as well and were thus long deficiencies extending to the left of Notch. In my X-ray series 7 out of 27 involve white, while in the spontaneous series only one out of ten is white, suggesting the working of natural selection in the case of the spontaneous series. When this is taken into consideration the two series show a striking similarity in the frequency distribution between the four groups differing in the length of the deficient segment.

DUNN, L. C., Columbia University, New York City: *Changes of dominance in the house mouse.*——The Brachyury (short-tail) mutation behaved as a dominant in the stock in which it was found by DOBROVOLSKAIA-ZAWADSKAIA. When outcrossed and backcrossed to several inbred stocks of *Mus musculus* and to *Mus bactrianus*, the expression of the mutation in the heterozygote was *decreased*, the degree of dominance modification depending upon the particular stock to which crossed. These results resemble those obtained with two stocks by GREEN. Additional observations do not indicate any marked effect either of modifiers or of heterosis upon the homozygote which regularly dies as embryo at about 11 days.——One normal stock (Bagg albino) which had been shown to have plus modifiers for Brachyury was tested with another short-tail mutation, *Sd*, of similar phenotype. In continued backcrosses to Bagg albino, the effects of *Sd* increased progressively, so that most heterozygotes are now tailless. No effect on homozygotes was noted. The same inbred stock thus modified two similar phenotypes in opposite directions. It is thus evident that dominance modifiers may have some specificity.

EIGSTI, O. J., University of Oklahoma, Norman, Okla.: *Effects of colchicine upon the nuclear and cytoplasmic phases of cell division in the pollen tube.*——The cytogenetical variations resulting from effects of colchicine upon nuclear and cytoplasmic phases of the division of the generative cell were studied by the microscopic slide method in *Tradescantia occidentalis* (n-6) and *Polygonatum commutatum* (n-20). A treated series consisted of the addition of colchicine to the cultural medium (sucrose-agar). In control cultures the double metaphasic chromosomes were arranged upon an equatorial plate from which the daughter chromosomes separate into their respective nuclei. The presence of colchicine inhibited the formation of an equatorial plate stage and prevented the complete separation of daughter chromosomes but did not preclude the

formation of nuclei or impede the development of pollen tubes. A variable number of nuclei was found in the pollen tube of the treated series. Cytological studies of the morphology and number of chromosomes present in nuclei formed thus revealed that both daughter chromosomes were present in the same nucleus. A detailed study of two nuclei in one pollen tube of *Polygonatum* disclosed that one nucleus contained 11 double chromosomes (22 daughter chromosomes) and the other had 9 double chromosomes (18 daughter chromosomes). Each nucleus formed in the presence of colchicine therefore is a cytogenetic entity unlike any other nucleus in the same pollen tube or unlike the nucleus formed after the usual division of the generative cell. The frequency of chromosomal breakage is higher in treated material which indicates that colchicine induces variations other than polyploid changes. The production of chromosomal breakage and the formation of nuclei containing both daughter chromosomes when pollen tubes are treated with colchicine provides the basis for multitudinous variations of a cytogenetic nature.

GILES, NORMAN, Harvard University, Cambridge, Mass.: *Spontaneous chromosome aberrations in Tradescantia.*——The types, frequency, and loci of spontaneous structural chromosome aberrations in a number of pure species and in the progeny of a natural species hybrid of Tradescantia have been studied at first post-meiotic mitosis. These aberrations are similar in most respects to chromosome alterations induced by low dosage X-rays in the developing microspore. This similarity suggested that the spontaneous breaks might be due to natural radiation. Considerable evidence is presented, however, to show that this is not the case. Localization of the breaks in the centromere region suggests that tortional strains occurring during the coiling cycle may be a major factor in causing spontaneous breaks. The frequency of spontaneous aberrations varied widely among individuals of the F_2 generation, indicating the possible genotypic control of the aberrations. The average percent of breaks in the hybrids was about three times that of the pure species, and it is suggested that this increase may result from the recombination of genetic factors following hybridization.

GLASS, H. B., Goucher College, Baltimore, Md.: *Differential susceptibility of the sexes of Drosophila to the effect of X-rays in producing chromosome aberrations.*——Treatment of mated females of *D. melanogaster* by X-rays provides simultaneous exposure of sperms and pre-meiotic eggs to identical doses. Offspring from eggs laid in the first five days following treatment yield approximately equal frequencies of lethal mutations in ♂ and ♀ germ-cells. 55 translocations were all produced in ♂ germ-cells. This disproportion is significantly greater than would be expected from the elimination of translocations in eggs undergoing meiosis. Inversions have been obtained from both ♂ and ♀ germ-cells. They are more frequent in the former, but the significance of the difference is not yet established.

GLASS, H. B., Goucher College, Baltimore, Md.: *Genetic identity of translocations of independent origin.*——The genetic identity of translocations associated with the dominant mosaic eye-colors bw^{V3}, bw^{V4} and bw^{V5} in *D. melanogaster* has been proved by obtaining reciprocal homozygotes for chromosome 2L·3L from one origin and chromosome 3R·2R from another. The genetic test here is probably more critical than the parallel cytological studies of salivary gland chromosomes. These three translocations were derived independently from X-ray treatments. Their identity indicates the presence in the chromosomes of definite weak spots especially subject to breakage and rearrangement.

GORDON, MYRON, New York Aquarium, N.Y.C.: *Gene frequencies and parallel variations in natural populations of seven geographical species of Mexican fresh-water fishes.*——The members of the genera Platypoecilus and Xiphophorus are divisible into a number of geographical species; each species of a genus occupies a distinctive river system. Most of them display, in nature, a parallelism of heritable patterns. Most variable is *P. maculatus* with its 127 phenotypic expressions, mostly recombinations of 15 genotypes. Some genes are rare (*Sb*, *Cô*, *Cò*, *Cs*, *Mc*, *Ct*, *T*), fairly common (*Sd*, *N*, *M*, *C*, *Co*) or quite common (*Sp*, *O*, *Cc*). In *P. variatus*, one subspecies has four genes (*Sp*, *M*, *C*, *Ct*), another has an extremely high percentage of one (*Sp*), and the third species has but one (*Sp*). *P. xiphidium* has four genes (*Sp*, *M*, *C*, *Ct*). Isolated populations within each species show a distinctive proportion of the genes. *P. couchianus*, furtherest north, has but the spotted gene (*Sp*). *X. montezumae* has a distinctive gene (*Sc*) in addition to *Sp* and *M*. *X. helleri* has the spotted gene rarely. *M*, *C* and *Ct* have been reported in the literature but are not represented in our wild caught specimens. A newly discovered pigmy Xiphophorus species has no patterns mentioned above. Collections made of *P. maculatus* in 1867, 1902, 1932 and 1939 indicate there is, in general, genic stability for a number of patterns. Comparative genetic studies of these patterns in all species is in progress.

GOWEN, JOHN W., Iowa State College, Ames, Iowa: *The structural significance of reproduction capacity in self-reproducing entities.*——In the evolution of complexity in living things the most striking phenomenon is the introduction of self-reproduction at some stage of the organization of high molecular weight proteins, between things called organic molecules and things called living. The characteristic reproductive capacity of a gene or a virus as well as size and mutative ability are common bonds of likeness between them. Inactivation of genes or of viruses, marked by loss of reproductive capacity, occurs in X-ray treatment. Tobacco mosaic virus of several strains may be inactivated exponentially by X-rays of 4 different wave lengths. By utilizing the quantum concept for the absorption of photoelectrons of known path lengths we may calculate the size of the vital volume of the molecule having to do with reproduction. This calculated volume is of 15,000,000 molecular

weight. Besides this volume, another portion of lesser but not as yet strictly defined size is occupied by elements capable of mutation without loss of reproductive capacity to the whole. The total of these two volumes presumably makes up the whole structure. An estimate of the molecular weight of this whole structure, 17,000,000 is furnished by the supercentrifuge experiment of SVEDBERG and ERICKSON-QUENSEL. A comparison of the two measurements shows that the portion of the molecule having to do with reproduction occupies the major part.

GREEN, E. L., and MCNUTT, C. W., Brown University, Providence, R. I.: *Bifurcated xiphoid, another effect of the short-ear gene in the mouse.*——A multiple recessive stock of short eared mice, *aabb*($c^{ch}c^{a}$)*ddpps*$^{e}s^{e}$, was found to have a formation of the xiphoid process of the sternum which differed from the normal rectangular type in that the posterior margin is deeply bifurcated. In F_1 from this stock by wild type (C_3H strain), the xiphoid processes were all rectangular. In the F_2 and the backcross generation, the bifurcated xiphoid reappeared in ratios characteristic of determination by a single mendelian recessive, though there was a slight but uniform deficiency of the recessive group. In every case, however, the progeny with bifurcated xiphoid were also short-eared. Since no crossing over between these two characters has yet occurred, it seems that bifurcated xiphoid process in this stock is caused by the same genetic mechanism which determines short-ears, and the morphogenetic processes which arrest the development of ears and bones of the cranium also arrest complete fusion of the bilateral sternal cartilages prior to their ossification in the medial plane to form normally the single rectangular xiphoid process. The deficiency of short-eared animals in segregating populations is probably caused by the often reported lowered viability of this genotype.

GREEN, M., and OLIVER, C. P., University of Minnesota, Minneapolis, Minn.: *The action of temperature and of non-allelic mutants upon heterozygous vestigial in Drosophila melanogaster.*——The frequency of wing notching in flies heterozygous for *vg* was not affected by 19, 23, 28, or 30°C, the frequency fluctuating between 0 and 1%. An increased frequency occurred with heterozygous *vg* in compound with dominant minutes. With $M(2)l^2$ the frequencies were 20, 65, 56, and 5 percent at the respective temperatures; and with $M(3)w$, 58, 80, 83, and 6 percent, with notching more extreme on the average. With each minute the highest frequency occurred at 23 or 28°. Several heterozygous mutant types were tested at 23° in compound with heterozygous *vg*. Each compound gave a notching frequency greater than that in the control, although in some cases only slightly higher. The frequencies varied from 1 to 82%, ranging in the order of increase: Controls; *In* $(3R)C$; *al S; Me; Pm dx;* $L^2M(2)l^2$; and $M(3)w$; but the degree of manifestation was not consistent with each increase in frequency. Some association between length of life cycle and frequency of notching is indicated, although that association seems not absolute. Temperature increase alone cannot account for the frequency

increase. Variations may be due to interaction of the mutant genes, possibly closely associated modifiers, with heterozygous *vg*, the temperature affecting the action of the modifying mutant. In general, males show a higher frequency of and also a more extreme notching. The notching varies from a slight to a beaded-like type.

HARNLY, MORRIS H.; Washington Square College, New York University, New York, N. Y.: *Experiments demonstrating no pupal critical period for wing size and form in four genotypes of D. melanogaster.*——Previous experiments with various genotypes for the vestigial locus have all shown a temperature effective period for wing size and form in the larva. It was thought that there might be another critical period for a different process of wing formation during the pupal stage, and that the second period might have a different critical temperature. Transferring dimorphos vestigial from 32°C at either 120 hours or puparium formation to 25° or 16° resulted only in a marked increase in mortality at the lower temperature. Our inbred vestigial stock was treated similarly and likewise gave negative results. Total development at 25° or transfers at puparium formation from 25° to 16° of the backcrossed vestigial-pennant stocks produced only flies with "nicked" wings. However, transfers at 96 hours to 16° gave the previously obtained frequency of normal and "nicked" wings. Tests of the original black vestigial-pennant stock produced at 16° only flies with normal wings, at 25° many with both wings normal, and at 30° some with normal wing margins. Furthermore, a "delta" was frequently present at 16° and extra cross-veins at the two higher temperatures. Preliminary reciprocal crosses between the two pennant stocks show that a sex-linked modifier affects the presence or absence of marginal nicks, and at least one or possibly two modifiers are associated with the appearance of extra cross-veins. A second critical period during the pupal stage was not found.

HEFNER, ROBERT A., Miami University, Oxford, Ohio: *Multiple alleles* (?) *in the inheritance of crooked little fingers.*——Earlier reports have designated hereditary crooked little fingers as Streblomicrodactyly. Subsequent investigations have revealed two additional types of hereditary factors resulting in crooked little fingers. These are given the descriptive titles of Minor streblomicrodactyly and Lateral streblomicrodactyly respectively. The former is shown from one family where it appears in 11 individuals. The latter is here shown from two families where many individuals are affected. The exhibit shows the three types of crooked little fingers by photographs, X-ray prints, and family charts. No family shown by these charts has any known relation to any other family in the group. The separate inheritance of each of these types of crooked little fingers shows Mendelian dominance over the normal condition. In some records the inheritance is that of an irregular dominant condition. How any of the streblomicrodactylous conditions behaves toward others in the series is not known since families where more than one of the deviations has been introduced have not been discovered. The location of such combinations would be

of decided genetic interest. The assumption of *multiple alleles* is at this time purely hypothetical.

HUESTIS, R. R., University of Oregon, Eugene, Ore.: *Tests for linkage in Peromyscus.*——Tests of four coat colors with one another and with flexed tail in *Peromyscus maniculatus* show no proof of linkage in any case. A double recessive recombination class was not obtained in over 400 F_2 individuals produced from a silver agouti-flexed tail cross but the ratio of individuals in obtained classes does not fit a theory of close linkage. The non-appearance this recombination class is not explained.

HUETTNER, ALFRED F.; Queens College, Flushing, N. Y.: *Differentiation of the gonads in the embryo of Drosophila melanogaster.*——After the primordial germ cells have been shifted dorso-posteriorly, they enter the amnio-proctodaeal invagination, where they remain loosely clustered at the posterior end of the diverticulum. They stay there impassive for approximately two hours without undergoing mitosis. During the sixth hour of incubation at 25°C, they make their way into the body cavity through the columnar cells of the gut diverticulum. This movement cannot be seen in the living material. From the study of fixed preparations, the impression is gathered that the movement through the gutwall is essentially amoeboid. As the hindgut diverticulum grows backward, the mesentodermal cluster of cells associated with the gut is carried dorso-laterally, where these cells will later contribute to the formation of the dorsal wall of the midgut. It is during this movement that the primordial germ cells are carried to their permanent position and become surrounded by mesentodermal cells. In ten to eleven hour embryos the gonads are formed. Not all the primordial germ cells which are carried passively into the amnio-proctodaeal invagination in the early embryo, are incorporated into the gonads. Some of them never pass through the gutwall into the body cavity, in others the migration is never completed, and in other instances they become entangled and lost in the yolk mass. Counts of the number of primordial germ cells in the primitive gonad show that approximately five to seven are grouped in some, whereas ten to thirteen are observed in others. In the light of KERKIS' report of the size differences in the gonads of newly hatched male and female larvae, these observations indicate that the sex of the gonads may, in all likelihood, be determined in the ten to eleven hour old embryo.

HUMPHREY, L. M.; University of Arkansas, Fayetteville, Ark.: *A preliminary report of the effects of inbreeding in cotton with special reference to staple length and lint percentage.*—In 1936 the inbreeding and hybridization method was introduced into the cotton improvement program at the Arkansas Experiment Station, and inbred lines were started in three varieties of cotton. Inbred lines for experimental purposes have been carried since 1930. In 1937 inbreeding was started in five more varieties and in 1938 in two more. All cotton varieties studied were found to be very non-uniform, particularly in fiber characteris-

tics. A highly significant negative correlation between staple length and lint percentage was found in five varieties, and no significant correlation was found in the remaining five. Cotton is largely self-pollinated, but there is enough cross pollination to introduce a certain amount of heterozygosity. Inbreeding rapidly segregates numerous types which become relatively very uniform after three to four generations, the inbred lines being much more uniform in all cases than the varieties from which they arose. Statistical studies of the lint percentages and staple length would indicate that the inbred lines become highly uniform after three to four generations of inbreeding, and no further significant increase in uniformity is evident up to eight generations. This would indicate the possibility of using three- or four-year inbred lines as inbred strains or varieties.

HUTT, F. B., and LAMOREUX, W. F., Cornell University, Ithaca, N. Y.: *A map for six linkage groups in the fowl.*——A map is presented illustrating the 23 mutations listed below, together with their wild-type alleles, and showing the approximate loci of 21 of these mutations in the six linkage groups now established for the fowl. These groups are numbered below in the order of their discovery and the numbers used have no relation to specific chromosomes. The approximate cross-over distances are given. (1) Sex chromosome: head streak—13.5—barring and slaty shanks (as yet unseparated in linkage tests) —27—brown eye—20—light down—16—silver—11—slow feathering. Locus of sex-linked gene for naked is not yet determined. (2) Rose comb—0.4—creeper—30—uropygial. (3) Crest—12.5—dominant white—17—frizzling, with fray tentatively assigned to this group. (4) Blue egg—5—pea comb—33 —marbling—46—naked neck. (5) Silkie—10—flightless. (6) Duplex comb—28—multiple spurs. In the sex chromosome arrangement and distances given, present what seems the best fit of all available data. In the second and fourth groups the distances actually determined are as given but the arrangement requires confirmation.

HUTT, F. B., Cornell University, Ithaca, N. Y.: *A new linkage group in the fowl.*——Genetic studies of the multiple spurs found in Black Sumatra fowls have shown this character to be an autosomal unifactorial mutation, incompletely dominant in heterozygotes but always easily distinguishable from the normal condition. The symbol *M* is proposed for the causative gene. Males have usually three spurs on each shank, but may have up to five. In adult females the mutation is clearly shown by three enlarged scales and an absence of the spur papilla usually found in females. It is classifiable in newly-hatched chicks regardless of sex with an accuracy of more than 98 percent. For these reasons the mutation is especially useful for studies of linkage. Tests for linkage with dominant white, rose comb, flightless and pea comb (each a marker for a separate linkage group) have all shown independent segregation of multiple spurs, thus apparently eliminating the gene *M* from the four autosomal linkage groups previously established. Further tests have shown it to

be linked with the gene *D*, for duplex comb, there being about 28 per cent of crossing over between the two genes. This establishes a fifth autosomal linkage group.

IRWIN, M. R., and COLE, L. J., University of Wisconsin, Madison, Wis.: *Interrelationships of the cellular characters of three species of Columbidae.*—— That part of the antigenic pattern of the red blood cells of Pearlneck (*Streptopelia chinensis*), not shared by Ring dove (*St. risoria*), has segregated into at least ten different characters, following backcrosses to Ring dove of the species hybrid and selected backcross birds. These characters have been designated as d-1, d-2, d-3 . . . d-11. Each of these individual components is produced by the action of one or more genes. The results of various tests show that a part of the specific antigenic complex of Pearlneck, as contrasted with Ring dove, is shared with still a third species, the Senegal (*St. senegalinsis*). Of the ten specific components of Pearlneck, only two (d-6 and d-11) appear not to be shared *in toto* with Senegal. It would be expected, then, in a cross of Pearlneck and Senegal, the offspring of backcrosses of the species hybrid to Senegal would be of four kinds in approximately equal numbers: viz., those whose cells contain (1) both d-6 and d-11, (2) d-6 alone, (3) d-11 alone and (4) neither of these constituents. Each of these four types of cells has appeared in the backcross offspring. The actual results obtained following a genetic test of the relationship between these particular species gives added confidence as to the accuracy of similar comparisons between Pearlneck, for example, and other species with which no hybrids have been obtained.

IRWIN, M. R., and CUMLEY, R. W., University of Wisconsin, Madison, Wis.: *Interrelationships of the cellular characters of species of Columba.*——Earlier studies have revealed that in contrasting cellular characters of two related species, some characters are shared by both species, and, in addition, each species contains cellular components which are peculiar to itself. At least a part of the characters which distinguish one species from another may in turn be shared by a third. In both doves and pigeons, these species specific substances have been found to be genetic characters which segregate in accordance with Mendelian laws, and which are transmitted as unit substances through species-hybrid and back-cross generations. In the present comparison of eleven species of *Columba*, some pairs of species were found to possess the major part of their antigenic components in common; and their specific characters were but infrequently shared with other species. In other contrasts, two species were seemingly more distantly related, having less antigens in common and sharing at least a part of their respective specific antigens with a majority, if not all, other species. All intermediate degrees of relationship were found. Apparently, no one species is composed of a combination of the antigens (genes) of two other species, and it is doubtful whether a combination of antigens in any number of species could account for all the antigens present in any one. However, the data regarding this question are as yet incomplete.

JEFFREY, EDWARD C., and HAERTL, EDWIN J., Harvard University, Cambridge, Mass.: *Nuclear fusions in relation to chromosomal structure and inheritance.*——The demonstration is illustrated by photomicrographs, microscopic preparations and drawings. These show the great advantages in connection with the study of chromosomal organization resulting from the study of the numerous examples of nuclear fusions, namely reproductive (diploid) and endospermal (triploid and quadruploid) presented by the Angiosperms. In all cases regardless of the number of chromosomes involved the organization of the post-fusional chromosomes is the same as in somatic cells, namely each chromosome contains two oppositely spiraled chromatids. It is thus clear that there is no microscopically visible result arising as a sequal to nuclear fusions, reproductive or otherwise. The facts emerging in the present investigation depend on the utilization of a greatly improved technical manipulation in connection with the fixation and staining of the chromosomes, which results for the first time in a clear revelation of their internal organization. It is now obvious that the structure of chromosomes of the categories somatic (sporophytic), meiotic, gametophytic and trophophytic (endospermal) is identical. In other words chromosomes may differ in number in the various categories of nuclei but not in organization.——This situation appears to necessitate a profound revision of cyto-genetical theories related to chromosomes, particularly the long held hypothesis of synapsis (syndesis) or chromosomal pairing in meiosis. The terms leptotene, zygotene, pachytene and diplotene also apparently can no longer be logically or accurately used. Chiasmatypy, so called, is as characteristically present in somatic, gametophytic and endospermal divisions as in those designated meiotic and consequently can apparently have no significance in connection with reproductive inheritance.

KAUFMANN, B. P., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Induced changes in chromosomes carrying inverted sections.*——The distribution of 278 X-ray induced breaks was plotted by use of salivary gland chromosomes of *Drosophila melanogaster* containing the dl-49 inversion in order to detect any possible reinversion and to measure break frequency in regions adjacent to the limits of the inverted section. No significant differences were detected between break distribution in these regions and in similar regions of chromosomes having the wild-type sequence of banding. However, data for the entire X chromosome showed that there exist certain intercalary regions of high break frequency, which probably contain heterochromatic material. If the original breaks leading to the inversion should occur in such intercalary regions, the possibility of reinversion would be increased.——As a corollary of this study, analysis was made of a series of induced changes to wild-type from rough^{est}, which is associated with a long inversion extending from 3C to near the spindle attachment region in the X chromosome. Reversion of rough^{est} is not infrequent, and five changes to wild-type have been analyzed. Two are reciprocal translocations between 2L and X heterochromatin; two are reciprocal translocations between 3R and X heterochromatin;

one involves transfer of the nucleolus-organizing region from the X to 3L. Obviously phenotypic reversion of roughest[3] may be associated with various types of chromosomal rearrangements, independent of reinversion, probably involving in each case a break or breaks in the proximal heterochromatic region of the X chromosome.

LAWSON, CHESTER A., Wittenberg College, Springfield, Ohio: *Sexual balance and the differentiation of aphid types.*——It is proposed that differentiation of aphid types is controlled by the opposing action of two forces (male and female) operative during development. If the male force dominates completely, a male develops. If the female force dominates completely, a gamic female develops. Incomplete dominance by the female force causes the development of the following types in order of decreasing female dominance: gamic-parthenogenetic female intermediate, wingless parthenogenetic female, wingless-winged parthenogenetic female intermediate, winged parthenogenetic female. Parthenogenetic females are sex intergrades or mosaics. Supporting evidence is found in the classification of differential structures into male and female characters and the correlation of these characters with aphid types. Male characters are wings, wing muscles, ocelli, many secondary sensoria on antennae, thin hind tibiae lacking sensoria, male reproductive system. Female characters are no wings, no wing muscles, no ocelli, few secondary sensoria on antennae, swollen hind tibiae with many sensoria, female reproductive system.

LAWSON, CHESTER A., Wittenberg College, Springfield, Ohio: *The probable mechanism controlling wing development in aphids.*——The germaria in embryonic winged and wingless aphids differ prior to time of determination of wings. Nurse cells in germaria of embryos destined to be wingless begin secretion of "nutrient" substance before wings begin development. This secretion presumably inhibits wing development. Nurse cells in germaria of embryos destined to be winged do not begin secretion before wings begin development. Lack of such secretion permits wing development.

LINCOLN, RALPH E., Iowa State College, Ames, and Cornell University, Ithaca, N. Y.: *Production and rate of mutation in Phytomonas stewartii by X-radiation.*——Broth cultures of *Phytomonas stewartii* were treated with X-radiation of 0.7A and 1.5A at intensities which reduced a culture of 100,000,000 viable cells to less than 1,000 viable cells within a period of 25 minutes. Inactivation curves for this organism are of the single absorption type——that is semilogarithmically linear. Variation was observed in colony type, colony color, virulence and mucoid characters. Mutation increases in proportion to exposure time. Under conditions where only one out of every 100,000 cells treated remains viable, the mutation rate is about 200 times higher than the natural mutation rate after growth in broth, or about 10,000 mutations per million treated cells as compared to 55 per million cells after

growth in broth. In general the same pattern and type of variation was found after X-radiation as after growth in broth. Most mutations observed are stable, hereditable and transmissible from parent to daughter cell, although certain stocks have been isolated in which it is impossible to stablize the variant type.

LOVE, R. MERTON, Cereal Division, Central Experimental Farm, Ottawa Canada: *A cytologically deficient speltoid of hybrid origin.*——Three bearded speltoids were found in F_2 Huron X R.L. 1005. Both varieties are bearded spring wheats. Progeny of the three speltoids totalled 36, of which 20 were normal and 16 speltoid. The 15 plants examined cytologically had 21 bivalents. Nine were normal cytologically and six were characterized by a heteromorphic bivalent, one member of which was deficient for the longer arm. Phenotypically, the nine plants were normal (in respect of the speltoid characters) and the six plants heterozygous for the deficiency were speltoids. No individuals homozygous for the deletion were obtained. Although the number of plants was small, the ratio seems to be characteristic of the C series type. The occurrence of the aberrant plants emphasizes the importance of hybridization in the origin of off-types. Breaks at the attachment region in unpaired chromosomes are a fruitful source of deletions which result in certain specific off-types in *vulgare* wheats. Hybridization, accompanied as it is by a comparatively high proportion of univalents in F_1 and some plants of later generations, plays an important role in the origin of speltoids and other off-types characterized by visible deficiencies. At the same time most, if not all, hybrids between *vulgare* wheats are heterozygous for one or more inversions (unpublished data). Fragmentation, caused by crossing over in the inverted regions, results in deletions. This too will lead to off-types, but small deficiencies may not be detected in wheats, where prophases are difficult to study. It is quite possible that A-series speltoids fall in this category.

MYERS, W. M., U. S. Regional Pasture Research Laboratory, State College, Pa.: *Tetrasomic inheritance in Dactylis glomerata.*——The regular occurrence of quadrivalents at meiosis in *Dactylis glomerata* indicates that tetrasomic inheritance should obtain. When self-pollinated, one plant produced normal green and chlorophyll deficient (lethal) seedlings in a ratio approximating 35:1. In progeny tests of 63 first generation inbred plants 16 produced only normal seedlings and 47 produced both normal and chlorophyll deficient seedlings. By means of X^2 the segregating families were separated into 32 approximating 35:1 and 15 approximating 3:1 ratios. Assuming that the parent plant was duplex (*AAaa*) with the gene located near the spindle fiber attachment, the expected ratio is 16.2 not segregating, 32.4 segregating 35:1, and 14.4 segregating 3:1. X^2 for fit of observed to calculated gave a value of P above .99.

LUCE, WILBUR M., University of Illinois, Urbana, Ill.: *Effects of oxygen on development and facet number in a bar infrabar strain of Drosophila melano-*

gaster.——Preliminary observations by MARGOLIS and by myself have indicated that oxygen has considerable effect on mean facet number and on the length of the period of development in bar strains of Drosophila. In the experiments here reported, an inbred strain of bar infrabar was subjected to treatment with oxygen for periods varying from 6 to 96 hours at different times throughout the egg, larval, and pupal stages. Continuous treatment throughout the entire egg stage or the entire pupal stage had little effect either upon the speed of development or upon mean facet number. Due to its lethal effect prolonged treatment could not be used during larval stages. Here treatment varied from 6 to 24 hours. Speed of development during larval stages was retarded by the treatment, the greatest retardation resulting from treatments during the first, second, and the first half of the third instars. Treatment during late third instar and prepupal stages had a slight retarding effect. The period of greatest effect on facet number was found to be from the 60th to the 80th hours of development, which corresponds closely to the temperature effective period for facet number in this stock. Oxygen has less effect on facet number in bar infrabar than it has in bar or infrabar. Thus the treated females showing the greatest effect had a mean of 31.18 ± 0.49 compared with controls at 24.88 ± 0.32.

MCNUTT, C. W., TETREAULT, T. E., and SAWIN, P. B., Brown University, Providence, R. I.: *Hereditary variations in the vena cava posterior of the rabbit.* ——In most mammals, including the rabbit, the posterior vena cava typically lies to the right of the dorsal aorta and passes dorsal to it at its bifurcation into the two common iliacs. This position in man, cat and pig is due to the persistence of the embryonic right supracardinal vein as the major part of the adult posterior vena cava, whereas deviations from it represent a substitution of one or another of the posterior cardinals, supra cardinals or cardinal collateral canals. A preliminary survey of these variations in five inbred families of rabbits and in crosses between them discloses approximately ten percent of so-called anomalies, among them being the marsupial type in which the vena cava, throughout its length and at its bifurcation, lies ventral to the aorta, representing a persistence of the embryonic cardinal collateral canals. Although the incidence of these variates in the total population is small and occurs in all families in which any numbers have been examined, it is found that ninety percent of them are either in inbred family V or in cross-bred matings to which family V contributes, and that within this family the most common variate is the marsupial type which in every case traces back two generations to the same male ancestor. The apparent genetic relationship between internal morphological characters peculiar to two separate but closely related orders of mammals suggests the possibility that further study of this variation may bear upon processes of phylogenetic as well as ontogenetic significance.

METZ, C. W., Department of Embryology, Carnegie Institution, Baltimore, Md.: *The nature of the chromatic granules in salivary gland chromosomes.*—— The range in pattern variation in selected regions is illustrated. For example,

in *Sciara ocellaris* one short region may appear in any of the following configurations: (1) Four conspicuous, similar transverse bands. (2) Three bands, the middle one much denser than the others. (3) Two transverse rows of conspicuous, block-like granules with concave sides, the granules of one row being much heavier than those of the other, and alternating with them in checker board fashion. (4) One row of block-like granules, with only faint traces of any additional structure. The block-like granules seem clearly to owe their concave sides and checker board arrangement to the presence of large achromatic droplets lying between them. The evidence here agrees with other evidence in indicating that the granules are chromatin masses which arise through distortion of the disks by the achromatic droplets, and that they have *per se* no genetic significance.

METZ, C. W., Department of Embryology, Carnegie Institution, Baltimore, Md.: *Spontaneous chromosome breakage during embryonic development in Sciara.*——In *S. reynoldsi* one chromosome pair regularly breaks into two segments during development in the salivary glands but not in ordinary embryonic cells. This provides additional evidence that the salivary gland chromosomes are highly modified structures. Here, as in the somewhat similar case described previously in *S. ocellaris*, the break probably occurs at the centromere region. It is postulated that two centromeres are present and that they separate spontaneously to effect the break. The phenomenon may have a bearing on the mechanism of evolutionary change in chromosome number.

MURRAY, M. J., Cornell University, Ithaca, N. Y.: *An F_2 study of interspecific characters.*——*Acnida tamariscina* (Nutt.) *Wood* native to the western prairie states hybridizes readily with *Acnida cuspidata* Bert. native to the coastal plain from Florida to Louisiana. F_2 and backcross progenies indicate that the major genetic differences between these two species are of three types: 1) On the basis of pollen abortion chromosomal dissimilarities are postulated; 2) Color and certain characters of the female flowers are inherited as simple Mendelian differences, while 3) such differences as leaf shape and size, stem size and character are inherited as quantitative characters. The chief differences involved in speciation in this particular example are of a quantitative nature presumably due to multiple genes. Hybridization is prevented only in so far as geographical and ecological barriers are effective.

MURRAY, M. J., Cornell University, Ithaca, N. Y.: *An attempt to obtain sex-linked mutations in a dioecious plant.*——A normal female plant of *Acnida tamariscina* (Nutt.) Wood was pollinated with X-rayed pollen. Seed from 474 different F_1 open-pollinated females were grown. No visible sex-linked mutations were found in over 250,000 F_2 seedlings and approximately 10,000 mature F_2 plants. This suggests that the Y chromosome has a full complement of allelomorphs. However, ten chimeras were obtained. These were chlorophyll changes associated with fifty percent bad pollen in the affected areas.

MYERS, W. M., and HILL, HELEN, D., U. S. Regional Pasture Research Laboratory, State College, Pa.: *The association and behavior of chromosomes in autotetraploid grasses.*——Three plants of *Dactylis glomerata* had a range in mean quadrivalent frequency in microsporocytes of 3.3 to 4.2, an average of 3.9; five plants of *Agropyron cristatum*, 3.4 to 4.2, an average of 3.7; and nine plants of *Arrhenatherum elatius* 3.4 to 4.6, an average of 3.8. The maximum number possible in each species is 7. The frequency of sporocytes with lagging univalents at anaphase I varied from 3.0 percent to 66.0 percent and generally corresponded in different plants with the frequency of their metaphase I nuclei showing univalents. In most plants there was an excess of laggards, indicating that some of them originated from other sources than metaphase I univalents. The lagging univalents divided equationally at anaphase I, and a majority of the half chromosomes were included in the daughter nuclei; the remainder formed micronuclei. The percentage of quartets showing micronuclei was correlated in the different plants with the frequency of lagging univalents at anaphase I. Observed numbers of micronuclei in eight plants varied from 13 to 52 percent of the numbers expected if all of the daughter half chromosomes from the anaphase I laggards formed micronuclei. One plant had more than was expected. The number and position of micronuclei in the quartets confirmed the assumption that most, if not all, of them originated from lagging and dividing univalents at anaphase I. Chromatin bridges and acentric fragments at anaphase I and telophase I of eleven plants studied indicated that each was heterozygous for one or more inversions.

NABOURS, ROBERT K., and STEBBINS, FLORENCE M., Kansas Agric. Exp. Station, Manhattan, Kan.: *Chromosome aberrations and viability in Apotettix eurycephalus Hancock.*——The X-ray induced translocations of chromatin have been reported, one with the exchange between first and fourth autosomes, with depletion in amount of chromatin for the former and augmentation for the latter (1933); the other between a first autosome and a sex-chromosome, with increase in chromatin and probably an extra traction fiber attachment for the latter. The autosome of this translocation was lost (1935 and unpublished). The three surviving aberrators are designated tentatively as 1. donor(a1), 2. receptor(a4), and 3. receptor(X). The genetic and cytological data correlate (Robertson, 1935, and unpublished). Zygotes carrying donor(a1), and otherwise normal, hatch but rarely survive to 3-4 instar when they are recorded (14 donor(a1): 2443 normals). Receptor(a4) are 13 percent, and receptor(X) 14 percent, less viable than controls. Donor(a1) +receptor(X) are 38.5 percent below normal. Donor(a1) +receptor(a4) +receptor(X) fall 17 percent below controls. The data comprise approximately 50 other items of comparison of 15,088 aberrators with 49,000 normal, and with each other. In practically all cases augmentation of chromatin is accompanied by less mortality among males than females, and vice versa when there is a depletion of chromatin. There is indication, but not yet definite proof, that the XO(absence of Y) chromosome condition in grouse locusts is responsible for the significantly

lower viability, and perhaps for the much smaller size, of the males among the controls in grouse locust breeding.

OFFERMANN, CARLOS A., University of Chicago, Chicago, Ill.: *Interference and the mechanism of crossing over.*——The present study discloses the general character of interference which, in view of the evidence offered, should be regarded as the expression of some intimate chromosomal feature uniformly manifest in normal and rearranged chromosomes. Numerous tests concerning the X-chromosome of *Drosophila melanogaster* led to the conclusion that in the euchromatic region of a chromosome, total obstruction of crossing-over at any point changes linkage relations according to a definite law: crossing-over will be restored with increasing distance from the point of interference at a rate approximated by $y = 1 - e^{-kx^4}$, where y represents the coincidence value at a distance of x map units from the point of interference. k has a fixed value for a given set of conditions depending upon age, temperature, irradiation etc. Thus for normal temperature (25°C) $k = 715$, and for high temperature (31°C) $k = 2500$, taking the age average for the first six days. The new formulation of coincidence in terms of distance opens up the possibility for universal comparison of different types of interference given hitherto only separate and incomplete consideration. Closely resembling pictures based on extensive tests for a given temperature are revealed for interferences resulting from transposed spindle attachments in homozygous and heterozygous condition, heterozygous rearrangements, combined rearrangements, and multiple crossovers in the normal chromosome. A substantiating check is offered by the parallelism displayed in each case by both the normal and the heat series. The unit concept thus established entails a series of conclusions concerning the mechanism of crossing over; linkage relations in new rearrangements will be predictable.

PARKER, MILTON M., Ohio State University, Columbus, Ohio: *The rôle of constitution in the emotionality of the adult albino rat.*——The principle objective of the present experiments was to determine the extent to which emotionality might be the characteristic product of a constitutional basis, and the extent to which it might be influenced by non-constitutional or environmental factors. The reasoning was that if a valid measure of emotionality were available, and were applied to a genetically heterogeneous stock of animals raised under uniform conditions, then intercorrelations obtained between emotionality measured in several different situations should afford an estimate of the variance attributable to constitutional as well as environmental factors. The measure of emotionality involved the ability of the rat to inhibit defecation in repeated presentations of an emotion-provoking situation. The emotionality score was equal to the number of trials which provoked emotional defecation until three successive trials failed to provoke defecation. Six emotion-provoking situations were constructed. Each situation administered a different type of intense stimulation and required a different mode of adjustment. The measure of adjustment, however, always consisted of the cessation of defeca-

tion. Standardization, on the basis of odd versus even trials, showed the reliability for each situation to be not less than 0.85. The fifteen intercorrelations (for each sex) obtained between various combinations of the six situations were all beyond the "one percent point" of significance, the coefficients varying from 0.65 to 0.85. The consistency and significance of the results suggest that emotionality is quite characteristic of the individual organism, although it may obviously be affected by environmental influences.

PATTERSON, J. T., STONE, WILSON, and GRIFFEN, A. B., University of Texas, Austin, Tex.: *The virilis complex in Drosophila.*——This complex can be broken down into two groups. One of these groups is more closely related to *D. virilis virilis* (Spencer) and includes, at the present time, the standard *virilis* from Pasadena, strains from New Orleans, China and Japan, and several Texas strains. The other group includes *D. virilis americana* (Spencer), *D. virilis texana* (Patterson) and at least one other Texas strain. The salivary gland chromosome analysis shows that all members of the *virilis* group are very similar; the members of the *americana-texana* group differ from the *virilis* group and among themselves. The metaphase chromosomes vary within the complex from five pairs of rods and one pair of dots to one pair of rods, a pair of dots and two pairs of V-shaped elements. Tests show that cross-fertility is very high in the *virilis* group, rather high in the *americana-texana* group, but low between the two groups. Genetic tests show that different cross-sterility factors exist in the several strains, and that at least some of these factors are recessive. There are several morphological and physiological differences between the several strains. However, sex ratios in the crosses are normal; both males and females are fertile, although there are some peculiarities in the fertility of F_2 and F_3 generation males bearing an *americana* or a *texana* Y-chromosome. The hybrids are usually normal. From these facts we infer that the genic balance and the sex-determination mechanism are much alike in the two groups.

PHILLIPS, H. M., Emory University, Emory University, Ga.: *Karyotaxonomy of Erythronium.*——1. *Preliminary survey of chromosomal number, morphology, and structure of developing microgametophyte of americanum.*—— In addition to morphological characters so often used, recent advances in systematics have introduced methods of observing additional natural phyletic indices. Chromosomal numbers, chromosomal morphology and structure, and genic changes have been used as a basis for verification or disagreement with systematists. Phyletic divergencies, families, genera, and species, have been shown to be correlated definitely with chromosomal changes. A preliminary investigation of *Erythronium americanum* has indicated the possibilities of this group for a detailed comparative study of hybridization, speciation, and phylogeny. An attempt will be made to determine the correlation existing between these phenomena and the karyology of the genus since the chromosomes

are large enough for a detailed analysis of structure and structural changes. Material is abundant, and the plants exhibit a number of interesting morphological variations. Material was collected from plants growing on Mt. Pinola near Atlanta, Ga. For permanent smears CRAF fixative was employed. Temporary mounts were made with Belling's iron-aceto-carmine method and with several modifications of this method. The *n*-number of *E. americanum* is 12. Counts were determined at late prophase, metaphase, and anaphase. A survey of polar view metaphase plates gave following results: 3 with 11 chromosomes; 187 with 12 chromosomes; 3 with 13 chromosomes; 1 with 24 chromosomes; 1 with 36 chromosomes. These counts from the microspore divisions indicate the possibility of a polyploid series within the species. Of 25 plants selected at random, however, no heteroploidy was encountered. The chromosomes of the dividing nuclei fall into two general morphology types; five chromosomes with submedian centromeres and seven with subterminal centromeres.

RICK, CHARLES, M., Harvard University, Cambridge, Mass.: *X-ray induced chromosome deletions in relation to mutation rate in Tradescantia.*——Small spherical fragments, independent of other chromosome aberrations, result from X-radiation of Tradescantia microspores. Evidence from size and shape indicates most of these to be interstitial deletions of ring structure. Their frequency within the dosage range of 100 r to 600 r indicates that they are conditioned mostly by 2 hits and that they are produced at random. X-ray treatment at 3°C induces significantly more deletions than the same dose at 30°C. The effect of X-radiation during the interkinesis preceding and the one following meiosis on the size of subsequently developed pollen grains was studied. Variability following both treatments is significantly greater than in controls. Variability at low temperature exceeds that at high temperature. Pollen size is interpreted as, at least in part, genetically self-determined. Since minute deficiencies are inseparable in genetic behavior from point mutations, the close parallel between fragment frequency and variability in pollen size suggests that the latter is a measure of mutation rate.

RIFE, D. C., and BOYE, C. D., Ohio State University, Columbus, Ohio: *The genetics of certain leaf variations in Coleus blumei.*——The following genetic variations occur in *Coleus blumei*. Factor *P* extends purple throughout the entire leaf, and an allele p^G results in solid green leaves. A third allele p, results in leaves with a purple pattern on the upper epidermis, surrounded by a green margin, and a green lower epidermis. Both *P* and p^G are completely dominant to p, while plants of genotype Pp^G are a brown gray color. A dominant factor *I* results in a dark green chlorophyll, and its recessive allele in light green chlorophyll. *P* and *I* segregate independently. Another dominant factor *A* results in chlorophyll throughout the entire leaf, and its recessive allele in leaves with albino midrib. Dominant factor *C* results in crinkly leaves, and its recessive allele in smooth leaves. *C* and *P* segregate independently. Another

dominant factor *S* results in deeply lobed leaves and its allele in shallow lobed leaves. All observed plants of genotype *Ss* are male sterile. A second factor *L* also results in deeply lobed leaves and apparently is lethal in the homozygous state. Colchicine produces marked effects when seeds are immersed for twenty-four hours in a one percent solution. Seeds obtained by selfing purple plants of genotype *Pp*, when treated with colchicine, produce solid black, solid red and patterned progeny. Presumably black plants are of genotype *PPPP*, red plants *PPpp*, and pattern plants *pppp*. Black plants are self-sterile.

RILEY, HERBERT P., University of Washington, Seattle, Wash.: *Interaction of genes for flower color in Nemesia strumosa.*——Gene *C* determines anthocyanin pigment on the inside of the corolla lips; *cc* plants are yellow if gene *W* is present, otherwise white. The anthocyanin gene is linked with the self-sterility alleles with a crossover value of 37%. *C* plants vary from purple to red according to whether *W* and unidentified modifying genes are present. The deep purple "eyebrow" above the stamens and the numerous deep purple dots on the lower part of the throat are due to gene *E; ee* plants have just a few pale lines above the stamens and almost no dots in the throat. Homozygous *f* plants have a variable number of small purple dashes of flecks on the lower lips; this is apparently a mutable gene. Plants homozygous for *bl* have a light blue color on the upper lips which is absent in *Bl* plants. The *ee* condition is expressed only if no other anthocyanin pigment is present; *ee* plants that have gene *C* or genes *ff* appear phenotypically like *E* plants. In *cc ww blbl* plants there is a blue margin about three millimeters wide on the upper lips if *E* is present; in *cc ww blbl ee* plants, the blue covers the entire upper lips. In plants with *C*, *ww* and *blbl*, the margin on the upper lips is bluish-purple and the blue sap pigment appears to be precipitated by the purple and collected as granules in the center of the cells.

SATINA, SOPHIA, and BLAKESLEE, A. F., Carnegie Institution, Cold Spring Harbor, N. Y.: *Morphological differentiation in chromosomes of Datura stramonium.*——Length and structural differentiation of chromosomes were studied at metaphase stage of division in young pollen grains of 2n and 2n +1 plants. Pollen was chosen because only the haploid number of chromosomes is present. Half of the pollen grains from a 2n +1 primary type contain n +1 chromosomes, there being two of one kind, so that the extra chromosome could be identified. Half of pollen grains from 2n +1 secondary types also contain n +1 chromosomes but the extra chromosome is a doubled half-chromosome. Thus the ends of this chromosome could be identified. Use also was made of prime types when secondary types were unknown. Each chromosome in haploid complement is V-shaped, the insertion region dividing it into two arms of unequal size, the amount of difference depending upon the chromosome involved. The longest primary chromosome (1·2) averages 4μ; the shortest (23·24) averages 1.5μ. The longest secondary chromosome (1·1) averages

4.5μ, the shortest (19·19) averages 1.8μ. Seven of the twelve chromosomes have satellites on one of the arms. Five of the satellites are small. Both the ·4 end of the 3·4 chromosome and the ·10 end of the 9·10 chromosome have large satellites. Only one of the secondaries (10·10) has as its extra chromosome a satellited arm doubled. Differences in width of chromosomes also were observed. Comparison was made between chromosomes of pollen and root-tip cells of haploids. The small satellites were more conspicuous in root-tip cells.

SAX, KARL, Arnold Arboretum, Harvard University, Cambridge, Mass.: *X-Ray induced chromosome aberrations and their subsequent behavior.*——Both chromosome and chromatid aberrations are produced by X-rays. The frequency of aberrations produced by single breaks shows a linear relation to dosage, while those dependent upon two breaks increase as the square of the dosage. Many of these aberrations produce single or double chromatid bridges at anaphase. When these bridges break new fusions of broken ends occur, although some of the broken ends appear to behave as normal ends.

SEARS, E. R., U.S.D.A., University of Missouri, Columbia, Mo.: *Monofactorially conditioned inviability of an intergeneric hybrid in the Triticinae.*—— Hybrids of *Aegilops umbellulata* with *Triticum monococcum* die at an early stage of growth, but when the same Aegilops species is crossed with *T. aegilopoides* (which on genetical and cytological grounds might be classified as a subspecies or variety of *T. monococcum*), the hybrids are viable. Results from both hybrid combinations are the same whichever direction the cross is made. Certain F_4 and F_5 derivatives of *T. monococcum*×*T. aegilopoides* have also produced viable hybrids with *Ae. umbellulata*. The same derivatives have been crossed to *T. monococcum*, and individuals from the resulting F_1 and F_2 hybridized with *Ae. umbellulata*. The results indicate that *T. monococcum* carries a single, mendelian factor which has a dominant lethal effect whenever it is combined with a genom from *Ae. umbellulata*.

SHANK, D. B., Iowa State College, Ames, Iowa: *Top-root ratios of inbred and hybrid maize.*——Inbreds and single crosses of maize were grown for six weeks on the three treatments (1) compost soil (2) washed river sand low in nutrients and (3) water cultures containing full nutrient solution. Completely randomized blocks were used. Top-root ratios based on dry weights were obtained and the data were subjected to an analysis of variance. The ten inbred lines tested showed differences that were highly significant. Their means ranged from 2.0 to 3.1. Top-roots ratios of four F_1 generations were either significantly lower or did not differ from their low-ratio parent while in all cases they were lower than their high-ratio parent. No differences between reciprocal crosses could be demonstrated. Treatment differences were highly significant, with water cultures showing the highest, and sand the lowest mean top-root ratio.

SHULL, A. FRANKLIN, University of Michigan, Ann Arbor, Mich.: *Adult intermediate-winged aphids not physiologically intermediate.*——If intermediates

are due to development starting as of one type, but finishing as of another, the combination of characters shown would depend on which type of development preceded and on the order of determination. The observed combination of characters do not agree as to the order of determination, nor as to the direction of change; there are exceptions to any assumed order for either direction. Since the structures from which intermediacy has so far been judged are fixed and unchanging in the adult, the combination of them reflects only the processes occurring in the embryo. The adult might well be of one type or the other, not intermediate, in its physiology; and if so, it should be of the type toward which development was changing in the embryo. A number of adult intermediates in two strains of aphids have been tested as to wing production in their offspring, and as to the occurrence of males and gamic females among those offspring. Winged and wingless adults differ in these respects in both strains tested. The experiments indicated that adult intermediates were essentially like the wingless adults, indicating that the direction of change must be from winged to wingless. This agrees with histological observations which show that all aphid embryos, including the ultimately wingless ones, have wing rudiments before birth. It probably must be assumed that the change in development is not steady, but fluctuating.

SHULL, GEORGE H., Princeton University, Princeton, N. J.: *The gene mutations of Oenothera Lamarckiana and its mutational derivatives.*——Photographic presentation of gene mutations found in cultures derived from *Oenothera Lamarckiana* during the past 21 years. The first demonstrated gene mutation in the *Lamarckiana* series of forms was mut. funifolia, discovered in 1918. Older probable gene mutations were mut. *brevistylis* found by DE VRIES in nature, and mut. *rubricalyx* found by R. R. GATES in a mixed culture. In the 21 years since 1918 the following fully authenticated gene mutations have been discovered in the cultures of the exhibitor: In the first linkage group, associated with the zygote lethals characteristic of *Oe. Lamarckiana;* in order of their discovery, have been (a) mut. *funifolia* (1918); (b) mut. *pervirens* (1920); (c) mut. *rubrifolia* (1930); (d) mut. *pollicata* (1932); (e) mut. *clusa* (1934); (f) mut. *petiolaris* (1935); (g) mut. *contracta* (1938). In the third linkage group, and thus generally free from any indication of linkage with lethal factors: (h) mut. *vetaurea* (1921); (i) mut. *supplena* (1923); (j) mut. *bullata* (1925); (k) mut. *acutifolia* (1929). Not in the first linkage group, but with other linkage relations still unknown: (l) mut. *acuminata* (1936); (m) mut. *recurva* (1939); (n) mut. *rotundifolia* (1939). The last three are strikingly manifested in young rosette stages and appear in typical Mendelian recessive proportions in progenies grown from self-fertilized *Oe. Lamarckiana*, but they have not yet been brought to bloom. With exception of mut. *recurva* they are vigorous types and their rosettes persist throughout the first season of growth. Among the new gene mutations of the first linkage group, mut. *contracta* also has not bloomed and probably will never bloom. Its linkage relations are obvious because it completely takes the place of seg. *decipiens* in progenies of certain mut. *erythrina* parents.

SINGLETON, W. RALPH, and CLARK, FRANCES J., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Cytological effects of treating maize pollen with ultra-violet light.*——A preliminary report of the cytologically observable effects of ultra-violet light has been published SINGLETON (1939). This is a continuation of the same study begun with DR. L. J. STADLER at the University of Missouri in 1937. Seed of five ears from treated pollen was grown (treatments and pollinations made by DR. STADLER). Three treatments with 2967 Å (56,000 ergs/mm²) and one with 2967 Å (64,000 ergs/mm²) produced 77 plants (from 84 seed planted). Of these there was one haploid, 19 plants with segregating pollen, 55 plants with normal pollen and two plants from which there were no collections. Seven of the segregating plants had no deficiency observable at pachytene; seven had observable deficiencies; one plant was very small and weak (no collection made) and was probably deficient; and four were not examined at pachytene. Three of the last four plants were normal at diakinesis but the fourth plant was not examined. Twenty-one plants (from 21 seed) were obtained from treatment with 2652 Å (28,000 ergs/mm²). Two had segregating pollen, one with a deficiency while the other, even though segregating empty pollen, had no observable change at pachytene. Deficiencies observed were on chromosomes 2, 4, 6, 7 and 10. Two plants had a deficiency on chromosome 4. In addition there was one translocation, 3-armed, the plant being deficient for parts of chromosomes 1 and 10. There was also an anomalous case of an apparent deficiency for the long arm of chromosome 6 with the short arm present in triplicate. This may have been a sectorial as pollen from this plant was not segregating.

SINNOTT, EDMUND W., and WHALEY, W. GORDON, Columbia University, New York City: *The developmental basis of inherited size differences in plant organs.*——Measurements of diameter of stem, petiole, leaf blade, ovary and mature fruit in various pure lines of Cucurbita and Lagenaria show that these organs are correlated in size, a large-fruited type having all its vegetative structures also proportionally larger than a small-fruited one. These differences between races are all related to differences in the volume of the apical meristem of the shoot, which is markedly larger in large-fruited types than in small ones. In F_2 of crosses between large-fruited and small-fruited races, this same correlation between size of fruit and size of vegetative organs is evident, indicating that size differences of the various organs are not independently inherited, but that in a given individual they all have the same genetic basis. This evidently operates by controlling the size of the apical meristematic region, from which all these organs develop.

SKIRM, GEORGE, Arnold Arboretum, Cambridge, Mass.: *Frequency of quadrivalent formation in Tradescantia tetraploids.*——In Tradescantia tetraploids derived from diploid gametes most of the chromosomes pair as quadrivalents, but in tetraploids produced by somatic doubling most of the chromosomes pair as bivalents. This behavior is attributed to structural heterozygosity of inter-

stitial regions of otherwise homologous chromosomes. In a tetraploid produced by gametic doubling homology is not complete and there is no preferential pairing between the four "homologous" chromosomes. In tetraploids derived from somatic doubling there is complete homology between the duplicated chromosomes so that preferential pairing favors bivalent formation rather than quadrivalents. In cross pollinated plants with some structural heterozygosity of the chromosomes an autotetraploid produced by somatic doubling should be able to survive in nature, because it would have bivalent pairing and relatively high fertility from the beginning.

SONNENBLICK, B. P., Queens College, Flushing, N. Y.: *The salivary glands in the embryo of Drosophila melanogaster.*——The salivary glands in *Drosophila melanogaster* are ectodermal in origin. Transverse sections of embryos incubated for eight hours at $25 \pm .3$ C show them as a pair of latero-ventral ectodermal plates situated immediately adjacent to the ventral nerve cord in the anterior portion of the embryo. The ectodermal plates invaginate, and as they increase in depth an external orifice remains. In these early glands differentiation into duct and gland proper has not as yet occurred. Ducts have appeared in twelve hour old embryos. The lengthening ducts approach one another and in sixteen hour embryos have united medially to form a common duct which enters the floor of the pharynx. Examination of preparations of several hundred embryos indicates that no mitoses ever occur in salivary gland cells. From the time of the appearance of the gland as paired ectodermal plates through the period of differentiation in the later embryo no cytoplasmic or nuclear division has been observed. Increase in the size of the glands is due, therefore, to the growth of the component cells in the plates which initially invaginate to form the glands.

SONNENBLICK, B. P., Queens College, Flushing, N. Y.: *Dominant lethals and Drosophila embryonic development.*——The cytology of dominant lethal genetic alterations induced by X-radiation and their effects on Drosophila embryonic development have not, hitherto, been reported. Zygotes whose development is initiated by gametes bearing such alterations die at various levels of development prior to eclosion, but particularly striking is the high death-rate among the embryos. Study of the embryos has indicated that (a) dominant lethals, which can kill when present in single dose, are primarily large chromosomal aberrations; (b) effects of the lethals are evidenced at different stages of development, even as early as the initial cleavages; (c) normal and abnormal figures may be noted side by side in a single egg; (d) dominant lethals may be induced in both sperms and ova; (e) nuclei with irradiated chromosomes can multiply but, occasionally, undifferentiated cellular masses may be observed within some embryos; (f) death occurs in the diploid phase of development.

STILES, KARL A., and DOUGAN, PAUL K., Coe College, Cedar Rapids, Iowa: *A pedigree of malformed upper extremities.*——This study traces skeletal anoma-

lies through three generations. The defect varies greatly throughout the family. There are two individuals which have only a malformation of the fingers, namely, a webbing between the thumb and index fingers and an inward curvature of the little fingers. Seven individuals exhibit malformations similar to the above two cases plus a partial fusion of the radius and ulna bones of the forearm. Three extreme cases show not only deformities including the first two types, but also defects in the shoulders and upper arms. In two of these three cases the humerus is only about one inch long. The investigation includes four generations of twenty-six individuals, twelve of them showing some variation of the malformation. This skeletal morbidity is not inherited as simple Mendelian dominant, but rather irregular dominance is indicated.

SWANSON, CARL P., Harvard University, Cambridge, Mass.: *Heterozygous inversions in Tradescantia.*——The frequency of meiotic inversion bridges was studied in a hybrid population of Tradescantia. Inversion bridges were correlated with chiasma frequency. No correlation was found between bridge frequency and number of terminal chiasmata, but a very high correlation was found between bridge formation and frequency of interstitial chiasmata. Heterozygous inversions appear to be frequent in interstitial regions, but they are not involved in crossing over when chiasmata are limited to the terminal regions of the chromosomes. Chiasma localization at terminal loci permits both structural and genetic heterozygosity of the chromosomes in Tradescantia species with few meiotic irregularities. Genetic control of the loci of chiasma formation in hybrid segregates results in crossovers and inversion bridges in interstitial regions heterozygous for inversions. Thus chiasma distribution and chromosome inversions serve as isolating mechanisms.

WALETZKY, EMANUEL, University of Wisconsin, Madison, Wis.: *The disproportionate interaction of bifid with other wing mutants in Drosophila melanogaster.*——The mutant bifid changes wing venation markedly, but not wing size. When bifid is combined with some mutants of the scalloped wing type, disproportionately small wings are formed. The reduction in wing size exceeds that expected as a result of simple additive effects. Disproportionately small wings have been found in the combinations of bifid with various Beadex alleles, scalloped, clipped, or vg^{nipped}. They are absent in the combinations of bifid with cut^6, $vg^{vestigial}$, miniature, and radius interruptus.

WALETZKY, EMANUEL, University of Wisconsin, Madison, Wis.: *Correlations between the manifold effects of Wrinkled in Drosophila melanogaster.*—— Reduced wings and abnormal black cephalic pigment are manifold effects of Wrinkled. The manifestation and the penetration of these two characters are correlated in flies heterozygous for Wrinkled. The smaller and more abnormal the wing, the greater the frequency and intensity with which the pigment appears. When the wings are practically normal, abnormal pigment is invariably absent. These relations also hold in $D+/+W$ and $D^3+/+W$ flies, which

frequently have practically normal wings. However, the penetration of the pigment character is not increased in $D+/+W$ flies whose wings are reduced by the presence of dumpy or miniature. W/W flies whose wings are very greatly reduced by $vg^{vestigial}$ show no abnormal pigment. Similarly, only a few Wrinkled vg^{nipped} and Wrinkled Beadex^{1} flies, with greatly reduced wings, show abnormal pigment.

WARMKE, H. E., and BLAKESLEE, A. F., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Polyploidy and the sex mechanism in Melandrium.*——Experimental polyploidy provides a new method for the investigation of the sex mechanisms in dioecious plants. The specific tools are: 1, the addition of entire chromosome complements; 2, the addition or subtraction of specific chromosomes by means of non-disjunction; 3, the synthesis of new types with intermediate sex chromosome constitution by segregation. In *Melandrium* (*Lychnis*) *dioicum* the diploid male may be represented as 2A+XY, and the diploid female as 2A+XX. The polyploid types 4A+XXXYY (male), 3A+XXY (predominantly male), 4A+XXXY (predominantly male), 4A+XXXXY (hermaphrodite) are therefore intermediate in sex chromosome constitution and give information regarding sex balance in this species. In the 4A+XXXY type it is possible to measure the chromosomes within a single cell and demonstrate that the Y chromosome is larger than the X. By utilizing a combination of the aids provided by polyploidy it is possible to locate the sex determining factors. Thus, the Y chromosome is shown to be male determining; it appears that the X chromosome is female determining, and that the autosomes play little if any role in sex determination. A 4n dioecious race, consisting of approximately equal numbers of 4A+XXXY males and 4A+XXXX females, has been established.

WATERS, N. F., and BRANDLY, C. A., U. S. Regional Poultry Research Laboratory, East Lansing, Mich.: *Studies in viability of poultry I. Inherent resistance to fowl paralysis.*——One of the objectives of the U. S. Regional Poultry Research Laboratory is to establish strains of definite genetic constitution with respect to resistance to so-called fowl paralysis. Experimental birds of ten different strains of White Leghorns were hatched from eggs purchased from breeders in widely scattered regions of the United States. Duplicate buildings and equipment on either side of a main Laboratory building are provided for the pathology and genetic studies. At the time of hatching, chicks of each strain from eight hatches were divided equally on a family basis. Approximately one-half of the chicks were placed on the genetic side and the other half on the pathology side, under a strict quarantine. Approximately two-thirds of the chicks on the pathology side were inoculated with blood from birds showing typical fowl paralysis, the others being held as contact controls. Examinations of birds from the genetic side affected with fowl paralysis as determinated by gross and preliminary microscopic observations show an incidence of 1.8 percent for the highest viable strain, while the least viable strain

had an incidence of 11.7 percent to November 1, 1939. Diagnoses of birds from the inoculated group showing fowl paralysis according to the criteria employed revealed an incidence of 11.8 percent for the most viable strain, and 30.4 percent for the least viable strain. Some families within each strain have shown definite resistance to fowl paralysis while other families have shown high susceptibility. There are a number of families in different strains that have had no mortality from any cause, while in some families from these same strains, mortality has been 100 percent. The data show results only through October 31, 1939. No definite conclusions of genetic significance can as yet be obtained from this material. There is the possibility that the resistance of a few of the families to fowl paralysis may be of genetic nature. There is, however, the possibility that such resistance may be acquired or that the birds may be latent or carrier cases of the disease.

WILSON, G. B., and NEBEL, B. R., New York State Agricultural Experiment Station, Geneva, N. Y.: *Changes in chromosome sensitivity to X-rays.*——Microspores of *Tradescantia reflexa* Raf. were rayed at various stages preceding first pollen-grain metaphase. Sensitivity was measured by the degree of chromosome alteration at first pollen-grain metaphase. Results indicate a positive correlation between the speed of mitotic development and the degree of response called "sensitivity." Several agents which either stop or retard division are being tested; first, for their own effect on chromosome and cell morphology and second, for their effects on sensitivity to X-rays as judged by comparisons with normal sensitivity curves.

WRIGHT, GERTRUDE, University of Toronto, Toronto, Canada: *Inheritance of form of flower in Linaria vulgaris Hill.*——Abnormal flowers found growing wild have afforded a starting point for this study. These are of two main types,——peloric and spurless-tubular. The extremes of both types show radial symmetry but flowers intergrading to the two-lipped normals are common. Pelorics and tubulars are recessive to the wild normals and the backcross gives in each case a 1:1 ratio. The F_2 of the cross normal × peloric is composed of 3 normal to 1 peloric. The cross peloric × tubular and its reciprocal produce normal flowers. That these synthetic normals differ genetically from the wild ones is shown by the crosses synthetic normal × peloric and synthetic normal × tubular. These result in the reappearance of both parental types in approximately a 1:1 ratio. Flower form seems therefore to depend upon at least two interacting factors. The wild normals may be designated *PPTT;* the pelorics *PPtt;* the tubulars *ppTT;* and the synthetic normals *PpTt.* If this is the case a new form is to be expected of the composition *pptt.* So far crosses of synthetic normals *inter se* have not revealed it. A few crosses of this type have yielded normals, pelorics and tubulars in the ratio 9:3:3 which suggests that the double recessive may be lethal. Further experiments are in progress.

ZELLE, M. R., and GOWEN, JOHN W., Iowa State College, Ames, Iowa: *On the origin of epidemic virulence.*——When an epidemic starts, a concatenation

of events occurs which enables a particular pathogen to sweep through a species. To particularize these events constitutes a most significant problem of disease resistance. If host and pathogen are closely integrated, one must change in susceptibility or the other in invasive power if epidemic disease proportions are reached. Following SCHOTT's experiments, our own have shown that susceptibility or resistance in different host populations may be markedly increased by 25 generations of controlled breeding. But these changes are too slow to explain the shift from a stable population to the unbalance of epidemics. The immediate origin seems rather in mutation of the pathogen's virulence. The selective force isolating and purifying the population to the invasive type would lie in the hosts.——Four experiments with mouse typhoid, *S. aetrycke*, support this analytical view. In experiments 1, 2 and 4 the host type did not materially influence virulence. The third experiment was markedly different. Bacteria, passed through resistant mice, suddenly became the most virulent experienced; those through the susceptible mice showed no such change. Reversal of hosts caused the very slightly virulent strain, previously inhabiting the susceptible mice, to gain virulence explosively in the third passage through resistant mice. The virulent strain from resistant mice, passed through the susceptibles, remained stable. The results thus far indicate infrequent pathogenic mutation with host selection as a cause of epidemic virulence, agreeing with work of WELLHAUSEN (1937) and LINCOLN (1939) on the corn bacterial wilt relationship.

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BARKLEY, FRED A. *Keys to the phyla of organisms: Including keys to the orders of the plant kingdom, (with glossary).* Pp. 40. Associated Student's Store. Missoula, Montana. 1939.

COOK, MELVILLE THURSTON. *Enfermedades de las plantas economicas de las Antillas.* Pp. 530. Figs. 171. Translated from English ms. to Spanish by José I. Otero. Universidad de Puerto Rico. Rio Piedras. 1939. $2.00.

MEYER, BERNARD S. AND DONALD B. ANDERSON. *Plant physiology: A textbook for colleges and universities.* Pp. 696. Figs. 150. D. Van Nostrand Company, Inc. New York. 1939. $4.50.

ROBINSON, E. R. *New pronouncing dictionary of plant names.* (Approximately 3000 names; brief definitions, including English names following Latin names of plants.) 10 x 15 cm. Pp. 64. Florists' Publishing Co. Chicago. Oct. 1939. 25 cents.

SCHEINFELD, AMRAM (assisted by Morton D. Schweitzer). *You and heredity.* Pp. xvii + 434. Figs. 76. Frederick A. Stokes Co. New York. Sept. 1, 1939. $3.75.

SIMPSON, GEORGE GAYLORD AND ANNE ROE. *Quantitative zoology: Numerical concepts and methods in the study of recent and fossil animals.* Pp. xvii + 414. Fig. 52. Tables 13. McGraw-Hill Book Co., Inc. New York. July, 1939. $4.00.

TINKLE, WM. J. *Fundamentals of zoology.* Pp. xvi + 492. Fig. 278. Zondervan Publishing House. Grand Rapids, Michigan. August, 1939. $3.00.

WHYTE, R. O. *Research on grassland, forage crops, and the conservation of vegetation in the United States of America.* Herbage Publication Series. Bull. 26. Imperial Bureau of Pastures and Forage Corps. Aberystwyth. Great Britain. Sept. (Published Oct. 7), 1939. 5 shillings.

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